# Development of the scientific requirements of an Environmental Management System (EMS) for the pearling (*Pinctada maxima*) industry

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**OBJECTIVES:**

1. To determine relevant scientific requirements for a pearl industry EMS
2. To determine if the benthic physical / chemical or ecological variables beneath established pearl farms differ from the surrounding environment.
3. To develop the PPA's capacity to initiate and co-ordinate strategic research.

**OUTCOMES ACHIEVED TO DATE**

This project has been a successful collaboration between pearl farmers, academic scientists and museum taxonomists and has given the scientific community greater access to remote regions of Australia, facilitating the description of new species to science. It has highlighted the inherent variability and abundant biodiversity of shallow water benthic communities in northern Australia. The study employed an exhaustively designed sampling regime incorporating three spatial scales (10's of metres, 1-5 km, >100's km) and random sampling through time. A multi-control sampling strategy was undertaken to give an estimate of the natural variability of the region and to test for benthic impacts at three pearl farms that have been in use for up to 40 years. Multiple lines of evidence all conclude that the variability in benthic conditions at the farms is within the bounds of the natural variability at the reference locations. The main mechanisms that influence the impact of shellfish aquaculture are considered to be; the farming method, the density of the cultivated shellfish (or stocking rate), the water depth of the farm area and the hydrographical conditions in the area (Danovaro et al 2004). All these factors favor the northern Australian cultured pearl industry and would contribute to the lack of a benthic footprint documented by this study.

The conclusion drawn from these studies is that current pearl oyster culture techniques in northern Australia have no detectable effect on the sediments of the lease sites. As ongoing or frequent benthic monitoring is logistically challenging and expensive in context of northern Australian pearl farms and cannot be expected to observe anything but natural variability it would not be a wise use of scarce industry funding to include benthic monitoring protocols in the standard EMS for this industry. If major changes to farming practice creates uncertainty in the future on this issue, or political climate requires revalidation of these findings, a further study such as this, conducted as corporate industry research, such as this project, could again test the issue.

**NON TECHNICAL SUMMARY:**

The pearl oyster (*Pinctada maxima*) aquaculture industry in the Kimberley region of Western Australia has been established for decades. However, the potential environmental impact of this aquaculture had not been investigated for this region before this study. Other aquacultures (such as some finfish and other shellfish) have caused eutrophication (nutrient enrichment) of coastal systems and caused changes to benthic macrofauna and sediments (e.g. mussel culture; Grenz et al 1990, Stenton-Dozey et al 1999, Mirto et al 2000). Prior to this study it was not known through evidentiary research whether or not pearl oyster aquaculture in the Kimberley had the potential to foul the benthic layer under the farms through the deposition of faeces and pseudo-faeces from the cultured oysters and fouling organisms, and the fallout of debris from the long lines that suspend the pearl oysters (O'Connor et al 1999, Yokoyama 2002, Gifford et al 2004).

Our investigation has found this does not occur in pearl oyster aquaculture in the Kimberley region. Over the past two and a half years we have sampled the sediments below three *Pinctada maxima* pearl oyster farms in remote regions of the Kimberley coast each of which had been in continuous use for up to 40 years and were selected on the basis of their long history of continuous use. Sediment core samples were taken to measure physico-chemical parameters and grab samples collected the benthic macrofauna (>1mm in size). The physico-chemical parameters measured included the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. These sediment variables were chosen because they have been identified as some of the most sensitive indicators of nutrient enrichment (Hargrave et al 1997). Each farm was compared to 4 control locations (total = 12 control locations) within the same region selected on the basis of being at least 1 km outside the boundaries of the lease area and having comparable depths, current regimes and sediment types. There was no indication of eutrophication (nutrient enrichment) at any of the three pearl farms. There were also no consistent differences in the benthic macrofauna below the pearl oyster farms when compared to control locations.

Tests were also carried out to assess the effect of closing down a pearl farm on the sediments and associated benthic fauna. A pearl farm that has been in continuous operation for >50 years (Otama pearl farm, Kuri Bay) was scheduled for closure and samples of sediments were taken from under the longlines before the farm was closed and two further sampling periods conducted after closure (1 year and 2 years after). For each sampling period, three reference locations were also sampled. This allowed a Before-After-Control-Impact (BACI) analysis to be performed. Results indicate that the sediments under the longlines before and after the farm closure were not different to those of the reference locations.

The international literature reviewed through this project suggests that the main mechanisms that influence the impact of shellfish aquaculture seem to be the farming method, the density of the cultivated shellfish (or stocking rate), the water depth of the farm area and the hydrographical conditions in the area (Danovaro et al 2004). All these factors, passive farming, low stocking densities, high current flows, naturally high sediment loads and relatively deep lease sites, favor the northern Australian cultured pearl industry and would contribute to the lack of a benthic footprint documented by this study.

These results suggest that, in terms of pearl industry expenditure on their Environmental Management Systems (EMS), the monitoring or attempt at management of benthic impacts should be a low priority, and even of no priority, unless the practices referred to above change dramatically. If needed at all in the future, monitoring for benthic impacts in this industry can be appropriately handled by periodic studies conducted as corporate industry research, such as this one, conducted every 10-20 years, or if industry practices change radically in terms of stocking density, or conceivably because in time the industry feels the results presented here need re-validating. If the issue of benthic impacts is shown to have become an issue in the future the industry can adapt their EMS to respond.

**KEYWORDS:** ***Pinctada maxima*, culture, benthic impact**

# Background

## The Potential for Benthic Impacts from Farming Bivalve Molluscs

The gold or silver lipped pearl oyster, *Pinctada maxima,* forms the basis of Australia's pearl oyster culture industry located on the Kimberley coast of northern Western Australia (Prince 1999, Fletcher et al 2006). No artificial feed or chemicals are required in the culture of pearl oysters. The primary potential impact of this industry was thought to be the deposition of faeces and pseudofaeces from the cultured oysters and fouling organisms, and the fallout of debris from cleaning fouling growth from the long lines that suspend the pearl oysters in panels (O'Connor et al 1999, Yokoyama 2002, Gifford et al 2004).

Pearl oysters and other bivalve aquacultures are suspension feeders that feed on suspended particles from the water column. They then produce biodeposits in the form of faeces and pseudofaecal pellets as a waste product. It is thought that these biodeposits are similar in composition to the natural sediments because they are derived from phytoplankton and suspended particles (Grant et al 1995). However based on studies into other shellfish aquaculture industries, it was unknown whether these biodeposits and shell debris might accumulate in the sediments below the long lines, potentially leading to organic enrichment and even eutrophication (a detrimental increase of nutrients such as carbon and nitrogen). Although using only high pressure water and brushing (no chemicals are used) the industry practice of cleaning the biofouling organisms off the oysters and longlines during the culture process potentially may have resulted in accumulation beneath the lease.

From experience with other types of aquaculture it is known that accumulation of biological debris below aquaculture leases can change the substrates by reducing oxygen content (Hatcher et al 1994), increasing nutrient loads and alter dependant benthic macrofaunal communities (Pearson and Rosenberg 1978, Kaspar et al 1985, Chamberlain et al 2001). Benthic macrofauna refers to the animals (greater than 0.5 or 1mm in size) that live or are associated with the sea floor and mostly comprises of worms, molluscs including snails, crustacea (e.g.

crabs and shrimps), echinoderms (seastars and brittlestars), fish, and other small animals.

The detection of aquaculture related impacts in the marine environment, especially in the soft sediments of inshore regions, usually involves testing for nutrient and organic enrichment of the sediments and a change in benthic macrofauna communities (e.g. Pearson and Rosenburg 1978, Grant et al 1995, Harstein and Rowden 2004). Benthic macrofauna are sensitive to organic enrichment levels perhaps undetectable via bulk chemical measures and can reflect an accumulation of impacts over time (Crawford et al 2002). Numerous studies on shellfish aquaculture have demonstrated that a change in benthic macrofauna communities is one of the most sensitive measures of organic enrichment (Gibson et al 2000, Krassulya 2001, Crawford et al 2002, Dernie et al 2003, Thompson et al 2003, Barnes et al 2006).

In some parts of the world, mussel farms have been found to alter the characteristics of the seabed sediments (Grenz et al 1990) in sheltered sites where biodeposits and shell debris have built-up at rates of up to 10cm/year resulting in changes to the seabed up to 20m beyond farm boundaries (Dahlback and Gunnarsson 1981, Mattsson and Linden 1983). This build up of mussel biodeposits can organically enrich the sediments under mussel farms (Castel et al 1989, Grenz et al 1990, Gilbert et al 1997) alterating the macrofaunal assemblages in the sediments (Mattsson and Linden 1983, Tenore et al 1985, Stenton-Dozey et al 1999, Mirto et al 2000, Christensen et al 2003, Giles et al 2006, Callier et al 2007). This alteration of benthic macrofauna can include a decrease in the number of individuals and lower species richness (Mattsson and Lindén 1983, Kaspar et al 1985, Chamberlain et al 2001, Callier et al 2007). It can also involve a dominance of opportunistic species at mussel farms compared to reference sites (Chamberlain et al 2001: Site 2, Callier et al 2007) or the dominance of deposit feeders (Stenton-Dozey et al 1999).

In contract, other studies have found no effect on sediment nutrients from bivalve aquaculture, nor any change in benthic macrofauna (Hatcher et al 1994, Grant et al 1995, Crawford et al 2003, Miron et al 2005, Goncalves da Costa and

Cunha Nalesso 2006). A study of cultured mussels at Twofold Bay, Eden NSW found there was no evidence of any ecological impact on the benthic macrofauna below the longlines (Lasiak and Underwood 2002). They attributed their findings to the large, relatively open coastal area of Twofold Bay and suggested that mussel farms located in sheltered, poorly flushed areas where there is little opportunity for the dispersal of wastes away from culture sites can create nutrient enrichment and associated sediment changes (Lasiak et al. 2006). Other studies that have detected no significant impacts of mussel farms have also suggested that oceanographic characteristics are responsible for the lack of impacts (Chamberlain et al 2001, Hartstein and Rowden 2004, Miron et al 2005, Goncalves da Costa and Cunha Nalesso 2006). Chamberlain et al (2001) demonstrated that for mussel cultivation, the site with tidal flushing had negligibly impacted benthos, yet the benthos at a site with little tidal flushing had significant impacts. When currents are not strong enough to transport biodeposited material, the depth of the oxygenated layer of the sediment decreases and bottom oxygen may be depleted, leading to anoxia of the sediment and the overlying water (Chamberlain et al 2001).

In a study investigating the effects of different hydrodynamic regimes on biodeposits from mussel aquaculture Hartstein and Rowden (2004) found that macroinvertebrate assemblages only differed between farm and reference locations at low energy sites. No differences were observed between farm and reference locations at the high-energy sites. The physico-chemical parameters of total organic matter and the amount of mussel shell debris best explained the pattern of changes in the macroinvertebrate assemblage composition in the two low-energy study sites (Hartstein and Rowden 2004). They deduced that there is a relationship between the hydrodynamic regime and organic enrichment of seabed sediments by mussel biodeposits which can then result in the modification of the macroinvertebrate assemblage.

In general, the differences between studies may be attributed to difference in site hydrodynamics, topography, background enrichment, sediment type and especially culture characteristics such as bivalve stocking density, shell size and depth of line deployment (Callier et al 2007). For these reasons the potential or

predicted impacts of pearl oyster aquaculture cannot be assumed nor extrapolated from the numerous studies to date assessing effects of mussel aquaculture, making necessary this study of the sediments below pearl farms in northern Australia.

## The Ecologically Sustainable Australian Pearling Industry

Public awareness and government policies regarding Ecologically Sustainable Development (ESD) in the marine environment have been evolving rapidly over the last two decades. An industry of long standing, the *Pinctada maxima* pearling industry developed most of its practices well prior to this interest in the management of the marine environment becoming widespread. In line with changing public perception and government policy with regard to the environment, the Pearl Producers Association Inc (PPA) has long recognized the need to pro-actively address and demonstrate the industry's environmental responsibilities and practices. In 1998 the PPA commissioned the report: "The environmental impact of pearling (*Pinctada maxima*) in Western Australia" (Enzer MEC 1998). That report described the general environment in which pearling occurs and the pearling activities that might potentially modify the environment and concluded that the environmental effects of the pearling industry were likely to be minor and the industry environmentally benign. The report also made suggestions regarding the implementation of environmental monitoring programs to formally assess the conclusions and advised on the possible components of an environmental code of practice for the pearling industry. It suggested that the objectives of a code of practice should include:

- Establishing procedures that enhance Australia's reputation for producing high quality pearls through the application of ESD principles;
- Ensuring that pearl farms operate in a manner acceptable to the public and other users of the marine environment; and
- Providing guidelines for use by industry to ensure best practice techniques are adopted.

The Enzer MEC report provided an important benchmark summary of the current industry. It highlighted what was known within the industry and provided the R&D subcommittee of the PPA with an opportunity to review current environmental issues for the industry.

In 2001, funded through the WA Fishing Industry Council (WAFIC) Industry Development Unit (IDU) and the Fisheries Research and Development Corporation (FRDC), the PPA commissioned the environmental risk assessment consultancy, International Risk Consultants – Environment (IRCE), to conduct an environmental audit and risk assessment of pearl culture in WA (Jernakoff 2002 – FRDC 2001/099).

The consultants undertook an;

1. Evaluation of current Pearl Industry practices and procedures,
2. Ecological Risk Assessment on pearl culture including a workshop,
3. Environmental information gap analysis, and an
4. Environmental management gap analysis.

They concluded that the key environmental issue for the industry is whether or not there are long-term environmental impacts from pearl culture (Jernakoff 2002). In keeping with the conclusions of Enzer MEC (1998) they found that the available evidence suggests the environmental impact of pearling is low, observing however, that there was scant scientific evidence to prove this point. Jernakoff recommended that a study should be undertaken to document whether this is in fact the case, and to quantify the extent to which pearling might change the natural environment and recommended initially focusing on four components:

1. The composition of the fouling growth cleaned from cultured shell;
2. The potential for modifying benthic habitat below pearl farms;
3. The disposal of grey water from vessels and shore camps; and
4. Monitoring interactions with protected fauna.

In terms of direct assessment of the potential impact of pearl aquaculture on marine benthos, comparatively fewer investigations have been undertaken to date. In 1998, Enzer MEC suggested that the major environmental effect of pearl aquaculture in the region was the returning to the sea of marine growth cleaned from pearl oysters. However, Enzer MEC suggested that because no chemicals are used in the cleaning process and the material returned is of marine origin, the impact is temporally and spatially widely dispersed. In general, Enzer MEC found the industry to be environmentally benign. However this report did not directly test these assumptions by collecting field data.

Prince (1999) conducted a sampling program inside and outside a pearl lease to investigate the effects of *Pinctada maxima* aquaculture in the Montebellos Islands in WA and found no impact of the pearl farms on the abundance and diversity of the benthic macrofauna community. Despite this finding, there was great variability in the fauna among individual sites, and control sites were not at comparable depths to lease sites. This study was also limited both spatially and temporally as it compared two sites within pearl farms to three nearby reference sites at only one period in time (March '99).

Another study was undertaken in Port Stephens, NSW (O'Connor et al 2003), which examined the effects of a *Pinctada imbricata* pearl farm on sediment physico-chemical characteristics (sediment carbon, nitrogen and phosphorus). This study was temporally replicated (n=6 sampling times), and compared five reference sites to one farm site. Sediment variables examined beneath the pearl lease (including total organic carbon, nitrogen and phosphorus) did not differ significantly from the reference sites over the sampling times examined. Despite these findings, the authors acknowledged study limitations and called for future assessments of pearl aquaculture to incorporate benthic faunal community analyses and a Before/After, Control/Impact or "BACI" design whereby the sampling starts before the establishment of a farm.

Yokoyama (2006) compared the impacts of pearl farming and fish cages (yellowtail and seabream) in Gokasho Bay, Japan. The pearl farms in this region use rafts of *Pinctada martensii* (not longlines as in Australia) which covered

79000 $m^2$ of the bay and produced 800 kg of pearls in 1995 when this study was undertaken. They sampled the sediments under the pearl and fish farms and within reference sites for 18 months at monthly intervals. They compared the macrobenthic fauna as well as the sediment nutrient loads (carbon, nitrogen sulphur and dissolved oxygen) in these sites and found that fish farming created a large impact on the macrobenthic fauna and sediments, whereas pearl farming caused fewer effects. The community structure at the pearl farm site was similar to that at the control site, although there were lower densities and species diversity at the pearl farm site. There were also more seasonal changes in the dominant species at the pearl farm compared to the control sites.

Fletcher et al (2006) compiled a comprehensive report on the contribution of the pearl oyster *Pinctada maxima* fishery to ecologically sustainable development (ESD) in Western Australia. This assessment examined the economic, social and environmental benefits and costs of the pearl oyster fishery but did not empirically test any potential environmental costs. Similarly, Environment Australia produced an assessment of the ecological sustainability of the management of the Western Australia pearl oyster fishery in 2003, revised and renewed in 2008, against guidelines set out in Commonwealth legislation (Environment Australia 2003 and 2008). However this did not undertake any monitoring or assessment of the potential impacts of the aquaculture operations.

Collectively, few studies with sufficient temporal and spatial replication have been conducted to date to reliably assess the potential effects of pearl aquaculture operations on marine benthos. For an industry of such importance to the Australian economy, this lack of evidence has been a direct threat as there has been an Increasing level of interaction between competing coastal resource users. While government and industry are generally supportive of pearl culture due to its expected low environmental impact, community opposition to other forms of aquaculture has been increasing nationwide, placing political pressure on decision makers. High quality scientific information about the actual level of impact by the pearling industry in the Kimberley was deemed essential for informing rational coastal management into the future. The importance of this

information is highlighted by the fact that at the time of initiating this project the WA Fisheries Department's Business Plan highlighted the key objective of the pearling subprogram as ensuring ecological and environmental sustainability, while Strategy 4 of Program 1 in the FRDC R&D plan was 'increasing and applying knowledge of the effects of non-fishing activities, including the effects of aquaculture, on marine ecosystems.'

In 2003 the PPA became one of two industry association partners in the NHT funded Seafood Services Australia Ltd (SSA) pilot program for developing Environmental Management Systems (EMS). Through that project SSA and the PPA worked closely with the MG Kailis Group to develop and implement a cost effective EMS template that can be implemented generally across the pearling industry. Of central interest to that process was the relative necessity to implement benthic monitoring programs as a routine element of a Pearl Industry EMS. The environment in which pearl culture takes place typically involves, extremely remote locations accessed only by air or sea, high tidal flow and high turbidity which increases the difficulty, costs and risks associated with first sampling benthic sediments and faunas, along with the expense of freighting samples, analysis, evaluating and reporting. The permanent impost of routine benthic monitoring often associated with an aquaculture EMS would not be borne lightly by the pearling industry. A central aim of this project was to inform the content of Environmental Monitoring Systems for pearl farms off Northern Australia with regard to the relative priority for incorporating benthic monitoring as a standard part of environmental monitoring in the pearl industry.

This project has resulted in a comprehensive study undertaken by a research team from University of Newcastle examining the influences of pearl farming practices on the benthic sediments and macrofauna of the surrounding marine environment of the Kimberley coast of northern Western Australia. The project aimed to redress the limitations of previous studies of this kind by including greater spatial and temporal replication of both farms and reference sites. Furthermore, when one of the oldest continually used lease sites (>50 years of use) that was an initial part of the sampling program was closed for logistical

reasons, the project was able to undertake a Before/After, Control/Impact or "BACI" study to test the effect of *removing* a pearl farm on benthos. It was impossible in a project like this, with its limited 3 year time frame, to sample these pearl farms before they were established but closure of this farm enabled investigation of the effects of removing an established pearl farm for 2 years after its removal.

The current study represents the most comprehensive assessment of the effects of pearl aquaculture on benthic physico-chemistry and benthic macrofauna communities undertaken internationally to date. It represents a proactive collaboration between individual pearling operators, the Pearl Producers Association and scientists to redress this knowledge gap to ensure best practice environmental management of pearl leases of the Kimberley coast, North Western Australia.

## Need

The pearl oyster culture industry needs to operate in an environmentally sustainable manner and have the supporting science for communication of this fact to the public at large. The PPA has been developing an Environmental Management System (EMS) for industry and has found the specific scientific requirements to underpin an EMS for this industry have been difficult to define due to the general lack of basic documented knowledge about the environment in which it operates. Both Enzer MEC (1998) and Jernakoff (2002) explicitly highlighted the general lack of information about the environment and ecosystems on which the pearl industry depends, and a paucity of knowledge about how the practices involved with pearl culture interact with the environment. In a climate of increasing interest over the use of the coastal zone, this lack of documented knowledge is a direct threat for an industry of such importance to the Australian economy. While it was considered likely that this study would produce similar results to other studies of bivalve culture which found little or no benthic impact (Crawford et al. 2003, Gifford et al 2004), the distinct nature of the pearling industry (*P. maxima*) in the Kimberley limited the

usefulness of making generalizations based on the results of studies of temperate systems.

Thus the need addressed by this project was to study the Kimberley pearling industry mariculture practices with the aim of producing evidence based information about the industry's interaction with the environment upon which it depends so heavily to produce the world's best pearls. The PPA's longer term need was to continue developing its capacity to initiate, manage and complete programs of corporate research with the aim of enhancing environmental management, pearl production and status within the market and the community.

# Objectives

1. To determine the relevant scientific requirements for a pearl industry EMS.
2. To determine if the benthic physical / chemical or ecological variables beneath established pearl farms differ from the surrounding environment.
3. To develop the PPA's capacity to initiate and co-ordinate strategic research.

# Methods

## Aims and Objectives of the Scientific Study

This study investigated the influence of culture of the pearl oyster *Pinctada maxima* on the benthic assemblages and sediment physico-chemistry of the Kimberley coast, Western Australia. As detailed in Appendix 4 samples of the benthic macrofauna communities under the pearl long lines were compared with samples from communities from independent reference locations. Physico-chemistry of the sediments was measured such as the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. These sediment variables were chosen because they have been identified as

some of the most sensitive indicators of nutrient enrichment (Hargrave et al 1997).

As described in Appendix 4 three pearl farms were selected that have been in continuous operation for between 10-40 years and located in separate embayments around the Kimberley coast. The pearl oysters are suspended in multiple pocket panels at 2-3m depth, held in place by a drop line attached to surface floating long lines. The sediments under these long lines were sampled and compared to the sediments taken from four reference locations within the same embayment or area (total of 12 reference locations). Investigators hypothesised that if the pearl farms are having an impact on the natural environment then there should be differences in the sediments characteristics (physico-chemistry and macrofauna communities) between the reference and farm locations.

Tests were also carried out on the effect on the sediments and associated benthic fauna of closing down a pearl farm. A pearl farm that has been in continuous operation for almost 50 years (Otama pearl farm, Kuri Bay) was scheduled for closure for logistics reasons. Sediment samples were taken under the longlines before the farm was closed and for two sampling periods after closure (1 year and 2 years after) and for each sampling period, samples of the sediments were taken from three reference locations. This allowed completion of a Before-After-Control-Impact study (BACI). Investigators hypothesised that if the farm was having an environmental impact on benthic conditions, it would be expected that the sediments under the farm would change after removal of the farm, relative to the condition of the reference locations.

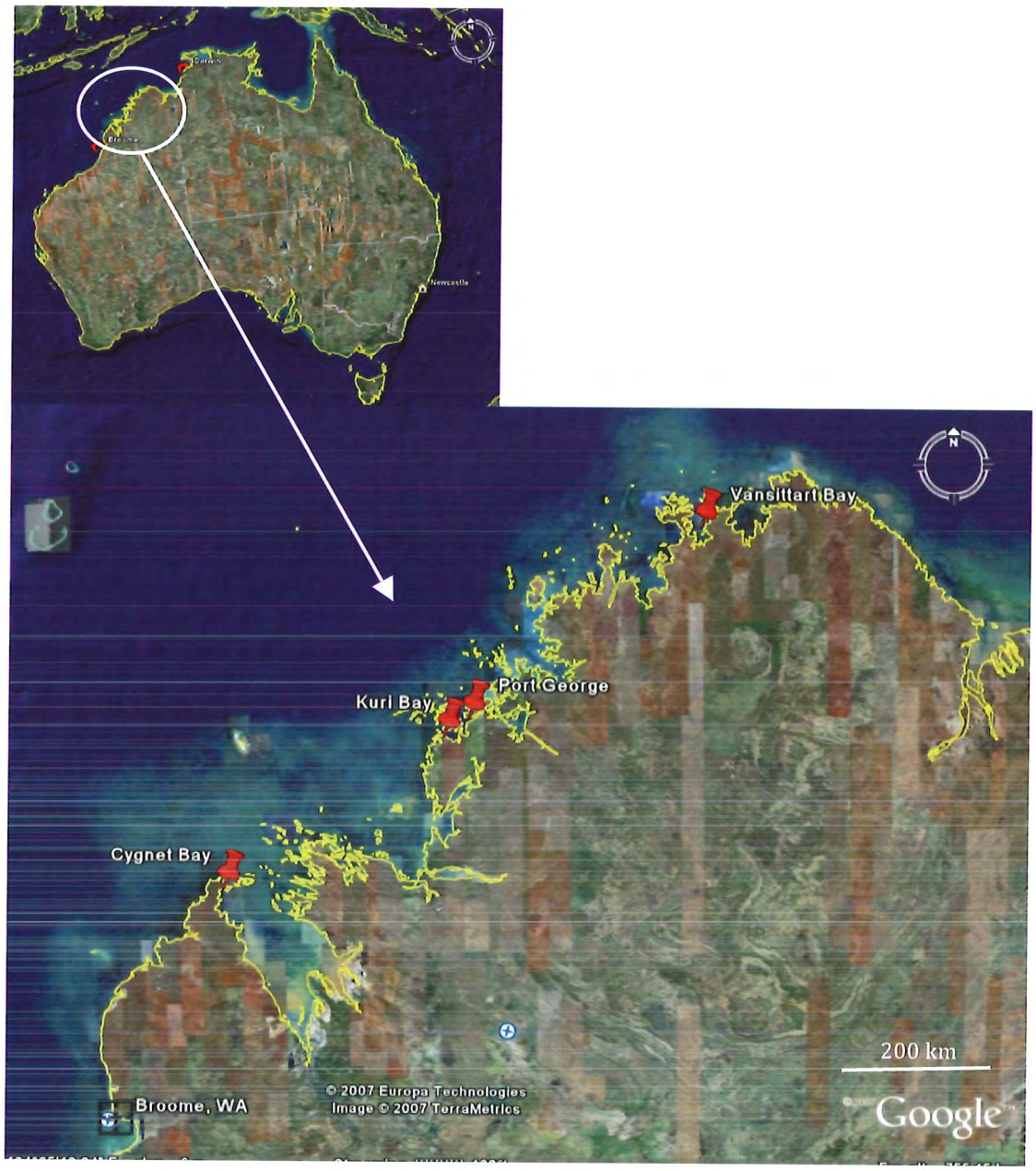


**Figure 1:** Map of Australia showing the Kimberley region and the study areas. The main study was conducted in Cygnet, Port George and Vansittart Bays and the BACI study was conducted in Kuri Bay (Image taken from Google Earth © Europa Technologies http://earth.google.com/).

## Study locations

As detailed in Appendix 4 the four pearl farms studied were located in the remote Kimberley coast of North Western Australia within the bays of Cygnet Bay (16°28'S, 123°02'E), Port George (15°23'S, 124°40'E), Kuri Bay (15°27'S, 124°31'E) and Vansittart Bay (14°01'S, 126°11'E) (Figure 1).

## Sampling design for the main study

As described in Appendix 4 the main study investigated the pearl farms in three bays, Cygnet Bay, Port George and Vansittart Bay over 10 sampling times. The bays were separated from each other by 100's of kilometres. The sampling occurred over 2 years (October 2006 to November 2008). At each bay, the condition of the benthos within the pearl lease (farm) was compared to four reference locations selected on the basis of being located at least 1km from the pearl lease boundary (and 2-8 nautical miles from the longlines), in similar water depths and having similar sediment types and current regimes. The design of this study was asymmetrical with the benthic conditions under three pearl farms compared to twelve reference locations. At each of these locations, there were 3 study sites that were spaced 50 metres apart (similar to the spacing of the pearl farm long lines). Within each site, 3 grab and 3 core samples were collected; a total of 9 grabs and cores for each location, and 45 for each farm. The grab and cores samples were collected ten times during the study (Grabs: Oct. '06, Jan., May, Sept. and Nov.'07, Feb., April, May, Aug. and Nov. '08. Cores: Oct. '06, Jan., Mar., May, Sept. and Nov.'07, Feb., April, May, Aug. '08). There were some exceptions to this sampling regime as some samples were lost in transit and the omission of some redox readings on two farms due to the temporary malfunctions of the redox probe during the study.

## Sampling design for BACI (Before, After Control, Impact) study

As described in Appendix 4 this study investigated the effects of removing a pearl farm (Otama pearl farm, near Kuri Bay) on the benthic conditions under the farm compared to nearby reference locations. This farm was closed down in November 2006 and all of the adult shell was removed from the longlines (some juvenile shell remained for a few months). The design of this study included three sampling periods; before the pearl farm was closed down (1-6 months before), 6-12 months after removal of the shell and 18-24 months later. These three periods are referred to as; before, one year after, and two years after. Within each sampling period there were two sampling times (nested). This study was asymmetrical with the benthic conditions under one pearl farm compared to three reference locations located at least 1km from the pearl lease boundary (and 2-8 nautical miles from the farm), in similar water depths and with similar sediment types and current regimes. At each of these locations, there were 3 study sites that were spaced 50 metres apart (similar to the spacing of the pearl farm long lines). Within each site, 3 grab and 3 core samples were collected, which is a total of 9 grabs and cores for each location, and 36 in total per sampling time. The grab and cores samples occurred six times during the study: before- May and Oct. 2006, 1 year after- January and May 2007, and 2 years- May and Nov. '08.

## Sediment grabs for Benthic Macrofauna

A Van Veen grab was used to collect the top layer of sediment (area = 0.1m$^2$, depth=10cm) which was gently sieved through a 1mm mesh on site. The material retained on the sieve was preserved in 5% formalin-saline containing Rose Bengal that stained the fauna pink. The sample was then sieved again back at the laboratory and sorted for the macrofauna that were then preserved in 70% ethanol.

The benthic fauna was then identified to the highest possible taxonomic level, usually genus or species although some fauna were identified only to order level. A low power dissecting microscope was used to count and identify the

macrofauna. Among Crustaceans, the most numerous group; the decapods, were identified to the species or genus level. Amphipods, isopods and tanaids were identified from the species to family level and the ostracods, stomatopods, mysids, and cumaceans were identified to order level. The polychaetes (segmented worms) were identified to the family level while the molluscs (including the bivalves) were identified to genus or species level. The echinoderms were identified mostly to genus level; the fish were identified to family level and the few sea spiders collected were identified to genus level. A small percentage of the benthic fauna included worms (flat, ribbon and acorn worms) sipuncilids, sponges, cnidarians and large forams which were not identified, however they were counted.

## Sediment cores for physico-chemical analysis

A universal gravity corer (68mm in diameter, Aquatic Research Instruments, Idaho USA) was used to collect the sediments for physico-chemical analysis. The corer collected over 20cm of sediment but only the top 5cm of sediment was used. The redox potential of the sediment was measured immediately after collection using a handheld pH-mV-Temp. meter (TPS Pty Ltd, Brisbane, Australia). The pH of the sediment was concurrently measured using pH indictor sticks. The sample was then placed into a sealed container, frozen and transported back to the laboratory where they were oven dried at 40°C and then ground. The physico-chemistry parameters measured in the laboratory from the sediment core samples were total organic matter, carbon, nitrogen, phosphorus, and carbonates.

The total organic matter was measured using the loss on ignition (LOI), furnace method (400°C, 16 hours). The total nitrogen and carbon content was measured using a LECO *Tru-spec*® CNS induction furnace analyser. Total phosphorus was determined photometrically after converting the organic phosphorus to inorganic phosphorus (furnace method: 550°C, 2 hours). The sample was then analysed using the ascorbic acid method for phosphorus analysis (Kuo 1996).

The carbonates were measured using the sequential loss on ignition method (Dean 1974).

## Univariate statistical analysis for the main study

The main aim of this study was to compare three farm locations with 12 reference locations so an asymmetrical analysis of variance (ANOVA) was used, the detail of which is provided by Appendix 4. The variables compared were the number of benthic species/families and individuals per grab and the sediment redox potential, total organic matter, total nitrogen, total phosphorus, total carbon and carbonates. The detailed design of this analysis is shown in Table 1 of Appendix 4. This type of asymmetrical ANOVA calculation has been previously employed by others when undertaking environmental impact assessment (e.g. Glasby 1997, Roberts et al 1998, O'Connor et al 2003).

## Univariate statistical analysis for the BACI study

As described in Appendix 4, a similar asymmetrical analysis of variance (ANOVA) was used to compare the sediments (and associated benthic fauna) over 3 sampling periods; before a pearl farm was closed down, soon after closing (within a year) and a longer time later (between 18-24 months later). The variables compared were the number of benthic species/families and individuals per grab and the sediment redox potential, total organic matter, total nitrogen, total carbon and carbonates. The detailed design of this analysis is shown in Table 2 of Appendix 4.

# Results / Discussion

## Main study: Benthic macrofaunal comparisons

As detailed by Appendix 4, more than two years of benthic sampling demonstrated that there was no evidence of any consistent change in the total number of benthic macrofauna taxa or individuals within soft sediments that might be directly attributed to pearl oyster longlines compared to reference locations. This outcome is particularly robust because of the rigorous sampling design that was employed to detect changes in benthic macrofauna over three spatial scales (sites, locations, bays) using numerous reference locations (n=4) in each bay and ten random sampling events in time. Too often an environmental impact of shellfish aquaculture (notably mussels) is demonstrated by studies that have only one control (or reference site) or one sampling time (e.g. Kaspar et al 1985, Tenore et al 1985, Grenz et al 1990, Stenton-Dozey et al 1999). Limited spatial or temporal sampling designs are inadequate to demonstrate any potential impact reliably (Underwood 1992) and for these reasons our study sought to undertake the most rigorous sampling protocol to date for assessing potential impacts due to pearl aquaculture.

As might be expected there was considerable natural variability of the benthic macrofauna among all location. Differences were observed in the assemblages of benthic macrofauna in the different bays (separated by 100's of km), as would be expected of different geographical regions. Larger scale processes such as biogeography, climate, and history probably drive this variability and contribute to these differences. However, there were no consistent differences in the benthic faunal assemblage under the longlines (at farms) when compared to the reference locations for all times. The fluctuations in benthic macrofauna found under the longlines at farms were within the bounds of what occurred naturally among reference locations. The reference locations were as different from one another as they were from the farm locations. The number of benthic macrofauna taxa, and their relative abundances within sediments underlying the farms fell within the range of natural benthic macrofauna variation observed at

the same spatial scales within reference sites. In fact, the greatest variability was observed at the within site level (50-150m distance) in comparison to the variability observed at the location level (1-5km distance). This can be typical of natural variability in benthic assemblages and corroborates other studies that show small scale variability in fauna can be greater than large-scale variability (Chapman et al 1995, Anderson et al 2005, Norén and Lindegarth 2005). This suggests that for the benthic species of this region, small-scale processes (such as competition between the species in the benthic assemblage, settlement and behaviour) may be more influential than other influences such as pearl farming activities. Therefore, in relation to small and large-scale processes, the farms had no detectable influence on the composition and abundance of benthic fauna in the soft sediments.

## Main study: Sediment physico-chemistry comparisons

When comparing among all bays (3 farms with 12 reference locations), the sediments under the pearl longlines did not exhibit the symptoms of nutrient enrichment or eutrophication observed in some other aquaculture industries (e.g. fish farms, Yokoyama 2002). Overall, the fluctuations of the sediment physico-chemistry under the longlines at the farms were within the bounds of what occurred naturally at the reference locations. Comparisons across all bays revealed no differences between what was observed at the farms compared to the reference locations. In fact, surprisingly very few of the samples taken from under the farms contained shell grit originating from the culture of pearl oyster. This is regardless of the fact that some of the farm sediment samples were taken soon after 'cleaning' of the longlines (removing epiphytic growth from oyster shells, panels and ropes).

## BACI study: Benthic macrofauna (effect of farm closure)

Despite the lack of 'before' monitoring in the main study, the project was however, afforded the opportunity to assess the potential effects of pearl aquaculture by monitoring before and after the *removal* of a pearl farm. The design of our Beyond BACI study (Underwood 1992) assumed that the removal of the oyster shell from the pearl farm longlines would cause sustained changes in the benthic fauna and conditions. This farm had been under constant operation for over 50 years so we would expect its removal to cause significant long-term changes *if* the farm had created some change or impact during its operation. The design of the Beyond BACI study allowed us to test for a 'pulse' disturbance, one that may occur soon after pearl oyster removal from the longlines.

The Beyond BACI study showed that the removal of the pearl oysters from the longlines did not have an effect on the underlying sediments and benthic fauna, when compared to the natural variability of the sediments at the reference locations. The changes observed between the benthic conditions six months before and then up to two years after the removal of the oyster shells were similar to the natural variability observed at the reference locations in this time. Although the assemblages of benthic macrofauna in this study changed significantly with time, there were no consistent changes in the benthic fauna assemblages that could be attributed to the removal of shell from the longlines.

## BACI study: Sediment nutrient levels (effect of farm closure)

The fluctuations of the sediment nutrient levels (total organic matter, carbon, nitrogen and carbonates) under the longlines at the farm were within the bounds of what occurred naturally at the reference locations, both before and after oyster shell removal. There were no differences between what was observed in the sediments at the farm compared to the reference locations, or any significant differences before and after shell removal at the farm. Similar to the main study,

the sediments under the pearl longlines did not exhibit evidence of nutrient enrichment or eutrophication.

These results from the Beyond BACI study concurred with the results of the main study in suggesting that pearl lease had no impact on the sediments or benthic fauna of the lease site.

## No dominance of indicator species of organic enrichment

Similarly the composition of the benthic fauna observed during this study support the notion that pearl leases have no impact on the benthic fauna within pearl leases. Benthic fauna has been used as an indicator of organic enrichment (from anthropogenic sources) particularly the Capitellid polychaetes, some Spionid polychaetes and gastropod molluscs. In fact 17 polychaete and 7 mollusc groups have been historically used as indicators of organic enrichment (Pearson and Rosenberg 1978), however none of these studies were from the Indo-Pacific region and so direct comparisons with our study are limited. Yet we did find that the benthic assemblage in the Kimberley, both at the pearl farms and reference locations, was very diverse and not dominated by one species or one group of taxa. In other studies of sediments affected by nutrient enrichment (or other environmental impacts), one or a few species or groups of taxa tend to dominate the sediments (Pearson and Rosenberg 1978, Stenton-Dozey et al 1999, Callier et al 2007). In particular for shellfish aquaculture, the use of ropes, racks buoys and lines is expected to have an immediate effect on local hydrography and provide a new substratum upon which other epibiota can attach and grow (Goncalves da Costa and Cunha Nalesso 2006). This can then potentially lead to a new or changed benthic fauna occurring in the sediments underneath the longlines.

Changes to polychaete assemblages are recognised as one of the best indicators of environmental impacts from aquaculture (Pearson and Rosenberg 1978, Hutchings 1998). Therefore the presence or absence of specific polychaetes in marine sediments can provide an indication of the condition or health of the benthic environment (Pocklington and Wells 1992). For example, some

polychaetes families, such as those belonging to the Capitellidae (i.e. *Capitella capitata*), Cirratilidae, and Spionidae families will dominate benthic communities in sediments experiencing excessive organic enrichment (Hutchings 2003, Giangrande et al 2005, Surugiu 2005, Cardoso et al 2007). Although some of these groups were collected in the sediments they were collected from the sediments of both farm and reference locations and potentially reflect a naturally occurring population of fauna. There were no differences in their numbers between farm and reference locations.

In contrast, some polychaete families can experience a decline in numbers during anthropogenic impacts. For example, the family Syllidae have shown to be a very useful indicator taxon in hard substrata as they are highly sensitive to pollution and disturbances, decreasing in numbers of species and individuals or completely disappearing in adverse conditions (Giangrande et al 2005). However in our study there were similar numbers of Syllids at the farms compared to the reference locations. Although we sampled in soft sediments and not hard substrata, the abundance of Syllids in the sediments under the longlines could suggest an absence of disturbance occurring under the pearl farms.

The mollusc species collected in our study reflected the normal fauna of tropical north Australia (Lamprell and Healy 1998, Lamprell and Whitehead 1992) and only one genus *Macoma* sp. was collected from the Kimberley that has been identified by Pearson and Rosenberg (1978) as an indicator of organic enrichment. However this mollusc was found in similar abundances at the pearl farms and reference locations.

## Comparison of our studies with other shellfish studies

In general, there is a lack of consensus regarding the environmental effects of shellfish aquaculture and this is not surprising given the different ecosystems and conditions that shellfish farms are located in. Furthermore the husbandry practices of each farm may be very different, and these can, in turn, influence potential effects of the farm on the natural environment. There are numerous

studies that suggest shellfish aquaculture can produce organic enrichment and alteration of benthic macrofauna (Mattsson and Linden 1983, Kaspar 1985, Tenore et al 1985, Stenton- Dozey et al 1999, Mirto et al 2000, Callier et al 2007) however half of these studies had limited spatial and temporal replication. In contrast, other studies suggest shellfish aquaculture to have little or no impacts (Hatcher et al 1994, Danovaro et al 2004, Goncalves da Costa and Cunha Nalesso 2006, Lasiak et al 2006) while others suggest that the conditions of the aquaculture and its environment can determine whether an impact occurs or not. For example, in Nova Scotia, one study found some biodeposition occurring under mussel lines compared to reference sites, but the sediments were not anoxic, and a diverse and active benthic community persisted regardless. They did find that the benthic community was influenced by the fallout of mussels from the farm lines, and this promoted the scavenger component of the benthic community. However, they did not find any enrichment of the sediment organic matter at the farm sites compared to reference sites (Grant et al 1995). Similarly, as mentioned previously, in New Zealand the influence of mussel farms was found to be dependant on variation in local current patterns (Chamberlain et al 2001).

Thus main mechanisms that influence the impact of shellfish aquaculture seem to be; the farming method, the density of the cultivated shellfish (or stocking rate), the water depth of the farm area and, as mentioned above, the hydrographical conditions in the area (Danovaro et al 2004). All these factors favour the northern Australian cultured pearl industry and could contribute to the lack of a benthic footprint documented by this study.

## Benefits and Adoption

The project reported here is the logical extension of two studies previously commissioned by the PPA; Enzer MEC (1998) WAFIC IDU project 00/05 and Jernakoff (2002) FRDC project 2001/099. These previous studies recommended that the PPA become pro-active in developing an ESD research capacity and a

culture of constantly improving environmental monitoring and management protocols. The PPA through partnership with Seafood Services Australia Ltd (SSA) pilot program developed an Environmental Management Systems (EMS) which can be implemented at each pearl farm site in the form of an Environmental Management Plan (EMP). The specific scientific requirements of an EMS for the industry have remained ill-defined due to a general lack of documented knowledge about the environment in which the pearl leases operate.

The results of this project suggest that, in terms of pearl industry prioritizing expenditure on their Environmental Management Systems (EMS); monitoring or attempts to manage for benthic impacts should be of low priority, and if needed at all in the future, monitoring for this issue can be appropriately handled by periodic studies conducted as corporate industry research similar to this project.

## Further Development

If needed at all in the future, monitoring for this issue can be appropriately handled by periodic studies conducted as corporate industry research. Periodic studies could be conducted every 10-20 years to revalidate these results if needed, or if industry practices change radically in terms of stocking density. If results indicate benthic habitat impact is shown to have become an issue in the future the industry can adapt their EMS to respond.

## Planned Outcomes

The Planned Outcomes from this project were:

- To document the actual level of impact being caused by the activity rated as the most significant potential environmental impact.

- To trial and prove cost effective techniques for monitoring the benthic environment in and around pearl leases

- To publish results of this research in refereed and semi-popular literature
- To inform the development of an EMS for the pearling industry
- To develop an organizational capacity to initiate and co-ordinate strategic research
- To contribute to the development of a culture of best practice and self-improvement with regard to ESD issues.
- To have pearl farms that are operating, and are seen to be operating, environmentally responsibly by the general public, other users of the marine environment, and the involved government agencies;

It was expected that the immediate output of this project would be the results of a tightly focused project to determine whether or not change can be detected in the benthic environment beneath three pearling leases, which by Australian Pearl Industry standards, have been used on a consistent and intensive basis for decades.

In terms of adopting the results from this project it was predicted that this would be achieved by gauging whether they inform the EMS being developed by the PPA. The project was expected to inform the EMS process about the relative importance of ongoing benthic monitoring, the type of technique and relevant timing of future benthic studies. Informing the EMS process can be considered the first wave of adoption of the results from this study.

A second longer term process of adoption was expected to be the broader discussion of the results within industry and more broadly through education within the general community. This process of adoption would be driven by the presentation of this projects results at workshops, meetings and conferences and through publishing written accounts in a wide variety of scientific, trade and semi-popular literature. This broader community education would provide an opportunity for the PPA to advance its reputation as an environmentally progressive industry.

## Actual Outcomes

• To document the actual level of impact being caused by the activity rated as the most significant potential environmental impact.

This study has been exhaustive in the design of the sampling regime; and employed three spatial scales and random temporal sampling. We have used a multi-control sampling strategy, to give an estimate of the natural variability of the variables measured. We have also used a Beyond BACI approach to investigate the effects of removing a pearl farm that had been established for over 50 years. These multiple lines of evidence all conclude that variability in benthic conditions beneath farms in the region are within the bounds of natural variability at other locations. This final FRDC report with the attached University of Newcastle report (Appendix 4), along with the series of scientific papers in preparation (see below) thoroughly document that current pearl culture techniques in northern Australia produce no measurable impact on the benthic habitat of pearl leases.

• To trial and prove cost effective techniques for monitoring the benthic environment in and around pearl leases

This project successfully trialed proven cost effective sampling techniques, which were then deployed through the project by the staff of the pearl farms. If another study of this kind was ever considered a priority again, the same techniques could be easily re-used. The major expense of this project was sorting of the samples, which contained many poorly known taxa. In the context of monitoring of the environmental footprint of the pearl industry the lack of any detectable impacts means this level of expense to measure natural variability is not justified.

• To publish results of this research in refereed scientific journals and semi-popular literature

UoN has several draft scientific papers being prepared for publication with tentative titles as follows:

1. Jelbart, J.E. Schreider, M and MacFarlane, G. The lack of impacts of pearl oyster *Pinctada maxima* aquaculture on marine benthos in Western Australia. *Aquaculture.*
2. Dixon, K.L., Jelbart, J.E. and MacFarlane, G. A lack of impacts from pearl oyster *Pinctada maxima* aquaculture on polychaete assemblages of the north-west Australian coast. *Marine Biology.*
3. Jelbart, J.E. Schreider, M and MacFarlane, G. A "Beyond BACI" approach to detecting a lack of impacts from pearl oyster *Pinctada maxima* aquaculture on benthic conditions. *Marine Ecology Progress Series.*

Two oral presentations at the Annual Conference of the Australian Marine Science Association were given by Dr Jane Jelbart (Appendix 3).

1. 2009, Adelaide, SA. *What are the impacts of pearl oyster aquaculture on marine benthos in WA?*
2. 2007, Melbourne, Vic. *Monitoring for potential impacts of pearl oyster aquaculture on marine benthos*

One student poster was presented by Kylie Dixon at the 2007 Annual Conference of the Australian Marine Science Association in Melbourne (Appendix 3).

1. *Assessing the Impacts of Pearl Farms on Polychaete Assemblages.*

<u>• To inform the development of an EMS for the pearling industry</u>

The failure to detect any differences in the sediments below pearl farms suggests that expenditure on monitoring sediments within an EMS would be a waste of the industry's resources. This is because this study shows that current culture practices has no impact on the sediments or benthic fauna below the pearl leases, while the natural processes cause the sediments and benthic fauna of the Kimberley region to be highly variable in space and time. In this situation benthic

monitoring programs run by pearl farms would monitor natural variation rather than the effect of the pearl leases, incurring an unnecessary level of expense for the farms.

• To develop an organizational capacity to initiate and co-ordinate strategic research

Through this project the PPA, supported by the expertise working for its member organisation, has demonstrated the capacity to engage and work with professionals and Universities to conduct field research in the difficult Kimberley marine environment.

• To contribute to the development of a culture of best practice and self-improvement with regard to ESD issues.

By pioneering the process of the PPA developing, implementing and completing corporate research projects into areas of corporate interest for the pearling industry this project has played a part in the PPA developing experience and expertise in applying a scientific approach to identifying issues and testing the basis of ESD issues for the industry. In this case existing practices have been vindicated as best practice in achieving the ESD outcomes required by the pearling industry and the community at large.

• To have pearl farms that are operating, and are seen to be operating, environmentally responsibly by the general public, other users of the marine environment, and the involved government agencies

The results of this study, vindicating existing practices as best practice with regard to the benthic habitat of the leases, fill a void in scientific information about this facet of pearl culturing in northern Australia and is being welcomed by the government agencies working with the industry in W.A., the N.T. and Commonwealth jurisdictions. Preliminary results from this project have already

been used by Paspaley Pearl's Pty in negotiating the right for pearl leases to co-exist within Marine Parks in the Northern Territory. These final results should provide further support for continued 'no-footprint access' to areas on northern Australian coastal habitats which are likely be considered of increasing value for conservation.

In the longer term the extension of this project will build from the experience, processes and expertise built by the PPA R&D committee through developing and supervising this proposal. This experience will provide the basis for the PPA becoming more pro-active in developing and undertaking a broader research agenda, and for developing within the PPA membership a culture of best practice and continuous self improvement with regard to ESD issues. The scientific results demonstrating no change to the benthos below pearl leases will foster a dialogue within PPA, and between the PPA members, the broader community and government agencies, about the environmental credentials of the pearling industry and the relative priority for further research or monitoring of the benthos compared to research or monitoring of other ESD issues. The extension process included in this project will foster this dialogue through its reference group meetings, industry meetings and workshops. Discussions that take place during those meetings will enable the PPA R&D committee to develop priorities and proposals for future collaborative research.

## Evaluating Originally Stated Planned Objectives:

1. To determine the relevant scientific requirements for a pearl industry EMS.

This project successfully conducted a detailed and rigorous study that strongly suggests that monitoring the benthos below pearl farms should not be a part of the pearl industry's EMS. This study demonstrated that benthic monitoring programs run by pearl farms would incur considerable expense upon farms and

simply monitor natural variation in the benthos rather than the effect of the pearl leases upon the environment.

2. To determine if the benthic physical / chemical or ecological variables beneath established pearl farms differ from the surrounding environment.

Over two and a half years this project exhaustively sampled the sediments below three *Pinctada maxima* pearl oyster farms in remote regions of the Kimberley coast. Sediment core samples were taken to measure physico-chemical parameters and grab samples collected the benthic macrofauna (>1mm in size). The physico-chemical parameters measured included the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. This project also tested the effect of closing down a pearl farm on the sediments and associated benthic fauna. These studies could find no consistent differences in the benthic macrofauna below the pearl oyster farms compared to independent control locations.

3. To develop the PPA's capacity to initiate and co-ordinate strategic research.

With this project the PPA has developed, implemented and completed a research project that addresses an area of corporate interest, the need for which, was identified and prioritised through ESD Risk Assessment Workshops (Jernakoff 2002). Through this project the PPA successfully developed the capacity to initiate and co-ordinate strategic corporate research, engaging and working with professionals and Universities to conduct field research in the difficult Kimberley marine environment. Through this process the PPA has developed experience and expertise in applying the scientific approach to identifying issues and testing their basis.

# Conclusions

Over the past two and a half years investigators sampled the sediments below three *Pinctada maxima* pearl oyster farms in remote regions of the Kimberley coast. Sediment core samples were taken to measure physico-chemical parameters and grab samples collected the benthic macrofauna (>1mm in size). The physico-chemical parameters measured included the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. These sediment variables were chosen because they have been identified as some of the most sensitive indicators of nutrient enrichment (Hargrave et al 1997). Each farm was compared to 4 control locations (total = 12 control locations) within the same region. There was no indication of eutrophication (nutrient enrichment) at any of three pearl farms studied in the Kimberley region. There were also no consistent differences in the benthic macrofauna below the pearl oyster farms when compared to control locations.

Investigators also tested the effect of closing down a pearl farm on the sediments and associated benthic fauna. A pearl farm was sampled that had been in operation for almost 50 years (Otama pearl farm, Kuri Bay) but scheduled for closure for logistical reasons. Sampled sediments were taken under the longlines before the farm was closed and two further sampling periods were conducted after closure (1 year and 2 years after). For each sampling period, samples were taken of the sediments of three reference locations. This allowed a Before-After-Control-Impact (BACI) analysis to be performed. It was discovered that the sediments under the longlines before and after the farm closure were not different to those of the reference locations.

These studies found no indication of eutrophication (nutrient enrichment) on any of the pearl farms which were selected on the basis of having extensive histories of continual use. Nor could this study find any consistent differences in the benthic macrofauna below the pearl oyster farms compared to independent control locations.

This study has been exhaustive in the design of the sampling regime; and employed three spatial scales and random temporal sampling. We used a multi-control sampling strategy, to give an estimate of the natural variability of the

variables measured. We also used a Beyond BACI approach to investigate the effects of removing a pearl farm that had been established for over 50 years. These multiple lines of evidence all showed that variability in benthic conditions beneath farms in the region are within the bounds of natural variability at other locations. In terms of observable impacts on benthic macrofauna and sediment physico-chemistry, current pearl farming practices in the Kimberley region can clearly be considered ecologically sustainable.

The main mechanisms that influence the impact of shellfish aquaculture seem to be the farming method, the density of the cultivated shellfish (or stocking rate), the water depth of the farm area and the hydrographical conditions in the area (Danovaro et al 2004). All these factors appear to favor the northern Australian cultured pearl industry and would contribute to the lack of a benthic footprint documented by this study.

The results of this project suggest that, in terms of the pearl industry prioritizing expenditure on their Environmental Management Systems (EMS) monitoring or attempts to manage for benthic impacts should be of low priority. If needed at all in the future, monitoring for this issue can be appropriately handled by periodic studies conducted as corporate industry research. Periodic studies conducted every 10-20 years could revalidate these results if considered necessary, or test the effect, if any, of a radical change to stocking density, in the unlikely event of that occurring. If results then indicated potential for benthic habitat impact to become an issue in the future the industry could then adapt their EMS to respond to that situation.

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# Appendix 1: Intellectual Property

The data collected, and the intellectual property developed this project's research, regarding sediment and benthic fauna of the Kimberley coast, are for public and scientific publication.

# Appendix 2: Project Team

**Project Team.**

| | |
|---|---|
| Brett McCallum | Executive Officer, Pearl Producers Association |
| Dr Scott Gifford | Post Doctoral Fellow, University of Newcastle |
| Dr Jane Jelbart | Post Doctoral Fellow, University of Newcastle |
| Ms Kylie Dixon | Honors Student, University of Newcastle |
| Dr Geoff MacFarlane | Senior Lecturer, University of Newcastle |
| Dr Maria Schreider | Lecturer, University of Newcastle |
| Dr Jeremy Prince | Marine Ecologist, Biospherics P/L |
| Dr David Mills | Paspaley Pearls P/L |
| Mr James Brown | Cygnet Bay Pearls P/L |

# Appendix 3: AMSA Abstracts

## Abstract for AMSA 2007

## Monitoring for potential impacts of pearl oyster aquaculture on marine benthos

**Jane Jelbart**[1], Scott Gifford[1], Kylie Dixon[1], Mary Greenwood[1], Maria Schreider[1], Jeremy Prince[2], Geoff MacFarlane[1]

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The pearl oyster (*Pinctada maxima*) industry in Western Australia has been well established for decades. However, there has been no investigation of its potential environmental impacts, until now. Pearl oysters have the potential to enrich the benthic layer under the farms through the deposition of faeces and pseudo-faeces. In addition, periodic husbandry practices remove epiphytic growth from the pearl shells on location, which may settle and accumulate under the farms. For these reasons, the benthos below three pearl oyster farms was compared to 4 control locations for each farm (total = 12 control locations). Sediment core samples were taken to measure physico-chemical parameters and grab samples collected the benthic macrofauna (>1mm in size).

At all three pearl farms, a preliminary investigation has found no significant differences between the benthic macrofaunal assemblages below the pearl oyster farms when compared to control locations. The macrofauna assemblage was also tested for a correlation with the sediment physico-chemical parameters to uncover any abiotic influences on community structure. The sediments at all three farms were not organically enriched as typical of some other shellfish aquaculture industries. This is of particular note as these farms are some of the longest operating in Australia (~50 years).

## Abstract for AMSA Poster 2007

# The impact of pearl aquaculture on polychaete assemblages in Western Australia

**Kylie Dixon**[1], Jane Jelbart[1], Scott Gifford[1], Maria Schreider[1], Mary Greenwood[1], Jeremy Prince[2], Geoff Macfarlane[1]

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Pearl aquaculture is well established on the northern west Kimberley coast of Western Australia, yet little is known in regard to the environmental condition of benthos below pearl leases. The principal environmental concern of shellfish aquaculture is the deposition of faeces and pseudofaeces produced by the cultured shellfish and the regular cleaning of associated fouling organisms from oyster shell. Biological waste may accumulate sufficiently to modify physico-chemical characteristics of the benthos below the pearl lease. This, in turn, may impact associated macrobenthic infauna. Benthic communities can influence surface productivity, alter the physical and chemical condition of the sediment and sediment-water interface, and transfer energy to higher trophic levels. Polychaete assemblages are widely employed as indicators of habitat condition and for the detection of human induced change. Three pearl leases were selected for this study as they have a relatively long history of farming activity. Specifically, the polychaete assemblage below each pearl farm was compared to 4 nearby control locations that were at a distance of 1 kilometre or greater from the lease location. Both uni- and mulitvariate analyses suggest that no consistent impact is evidenced at pearl leases in terms of polychaete assemblages. The farm locations exhibited similar abundance and diversity of polychaete taxa when compared to control locations.

## Abstract for AMSA 2009

# Does pearl oyster aquaculture have an impact on marine sediments and benthic fauna in Western Australia?

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The pearl oyster (*Pinctada maxima*) aquaculture industry in the Kimberley region of Western Australia has been established for decades. However, the potential environmental impact of this aquaculture has not been investigated for this region until now. Pearl oysters may also have the potential to enrich the benthic layer under the farms through the deposition of faeces and pseudo-faeces. Other aquacultures (such as some finfish and shellfish) have caused eutrophication of the marine sediments and a concurrent change in the benthic assemblages. However, our investigation has not found this to occur in pearl oyster aquaculture.

Over the past two and a half years we have sampled the sediments below three pearl oyster farms in remote regions of the Kimberley coast. Sediment core samples were taken to measure physico-chemical parameters and grab samples collected the benthic macrofauna (>1mm in size). Each farm was compared to 4 control locations (total = 12 control locations) within the same region. At all three pearl farms there were no indications of eutrophication. There were also no consistent differences in the benthic assemblages below the pearl oyster farms when compared to control locations.

In this presentation we describe the biodiversity of the region, including the natural variability and connectivity of the benthic assemblages. We also attempt to explain why some of this variability occurs in the region and the spatial scales of this connectivity. This project has increased our knowledge of the distribution and abundance of benthic fauna in the Kimberley region. It has been a successful collaboration between pearl farmers, academic scientists and museum taxonomists. The project has also given the scientific community greater access to remote regions of Australia and facilitated the description of new species to science.

# Appendix 4: University of Newcastle Report

# THE INFLUENCE OF PEARL OYSTER FARMS ON BENTHIC PHYSICO-CHEMISTRY AND MACROBENTHIC COMMUNITIES OF THE KIMBERLEY COAST, WESTERN AUSTRALIA

## A TECHNICAL DOCUMENT PREPARED BY THE UNIVERSITY OF NEWCASTLE FOR THE PEARLING PRODUCERS ASSOCIATION

SCIENTIFIC STUDY FOR FISHERIES RESEARCH AND DEVELOPMENT CORPORATION PROJECT 2005/044


## *Disclaimer*

In undertaking this work the authors have made every effort to ensure the accuracy of the information used. Any recommendations made in the report are done in good faith and we take no responsibility for how this information and report are used subsequently by others. Note also that the views expressed and recommendations provided in this report do not necessarily reflect those of the persons or organisations that have contributed their views or other materials.

## *Citation*

Jelbart, J.E., Schreider, M. and G. MacFarlane (2009). The influence of pearl oyster farms on benthic physico-chemistry and macrobenthic communities of the Kimberley coast, Western Australia. Technical Document prepared by University of Newcastle, NSW for Pearl Producers Association. Scientific study for FRDC Project 2005/044.

## *Brief*

During May 2006 to November 2008, researchers from the Ecology and Ecotoxicology Laboratory at the University of Newcastle conducted field sampling at four pearl farms nominated by the Pearl Producers Association (PPA) to gather primary data which would function as the scientific study for FRDC Project 2005/044 – Development of the scientific requirements of an Environmental Management System (EMS) for the pearling (*Pinctada maxima*) industry. This document outlines the results of this field work and subsequent laboratory analysis and takes the form of a technical document prepared for the use of the Pearl Producers Association.

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## EXECUTIVE SUMMARY

The pearl oyster (*Pinctada maxima*) aquaculture industry in the Kimberley region of Western Australia has been established for decades. However, the possible environmental impact of this aquaculture has not been investigated for this region until now. Pearl oysters may have the potential to foul the benthic layer under the farms through the deposition of faeces and pseudo-faeces from the cultured oysters and fouling organisms, and the fallout of debris from the long lines that suspend the pearl oysters (O'Connor et al 2003, Yokoyama 2002, Gifford et al 2004). This organic waste and debris could accumulate in the sediments below the oyster long lines and potentially lead to organic enrichment and even eutrophication (a detrimental increase of nutrients such as carbon and nitrogen). Other aquacultures (such as some finfish and other shellfish) have caused eutrophication (nutrient enrichment) of marine sediments and a concurrent change in benthic macrofauna (e.g. mussel culture; Grenz et al 1990, Stenton-Dozey et al 1999, Mirto et al 2000). Benthic macrofauna are sensitive to organic enrichment levels perhaps undetectable via bulk chemical measures and can reflect an accumulation of impacts over time (Crawford et al 2003). However, our investigation has not found this to occur in pearl oyster aquaculture in the Kimberley region during this study.

Over the past two and a half years we have sampled the sediments below three *Pinctada maxima* pearl oyster farms in remote regions of the Kimberley coast that have been in continuous use for up to 40 years. Sediment core samples were taken to measure physico-chemical variables and grab samples collected the benthic macrofauna (>1mm in size). The physico-chemical variables measured included the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. These sediment variables were chosen because they have been identified as some of the most sensitive indicators of nutrient enrichment (Hargrave et al 1997). Each farm was compared to four control locations (total = 12 control locations) within the same region. At all three pearl farms there was no indication of eutrophication (nutrient enrichment). There were also no consistent differences in the benthic macrofauna below the pearl oyster farms when compared to control locations.

We also tested the effect of closing down a pearl farm on the sediments and associated benthic fauna. We selected a pearl farm that has been in operation for almost 50 years (Otama pearl farm, Kuri Bay) which was scheduled for closure. We sampled the sediments

under the longlines before the farm was closed and for two sampling periods after closure (1 year and 2 years after). For each sampling period, we also sampled the sediments of three reference locations. This was a Before-After-Control-Impact study (BACI). We found that the sediments under the longlines both before and after the farm closure were not different to those of the reference locations.

This study has been exhaustive in the design of the sampling regime; and employed three spatial scales (10's of metres, 1-5 km and over 100's of km) and random temporal sampling. We have also used a multi-control sampling strategy to give an estimate of the natural variability of the region. These multiple lines of evidence all conclude that variability in benthic conditions beneath farms in the region are within the bounds of natural variability at the reference locations. Furthermore, this project has been a successful collaboration between pearl farmers, academic scientists and museum taxonomists. The project has also given the scientific community greater access to remote regions of Australia and facilitated the description of new species to science.

## RATIONALE

Few studies to date have directly considered the potential impacts of pearl oyster farming on the marine benthos (but see Enzer MEC 1998, Prince 1999, Jernakoff 2002, Yokoyama 2002, O'Connor et al 2003, Fletcher et al 2006). Although a number of studies have established impacts on benthic systems from other bivalve aquacultures, namely mussel aquaculture (e.g. Hatcher et al 1994, Grant et al 1995, Crawford et al 2003, Miron et al 2005, Lasiak et al 2006) it cannot be assumed that pearl oyster aquaculture may exhibit the same impacts due to inherent differences in stocking rates, molluscan filtering and biodeposition rates, removal practices of biofouling organisms from oyster shells, other husbandry practices and farm locations. Wells and Jernakoff (2006) highlighted the dearth of evidence to support or reject the claim of detrimental effects to benthos from pearl aquaculture initiatives and suggested that further research investigating the environmental performance of the pearl industry is necessary for best practice management of pearl leases.

For these reasons we have undertaken a comprehensive study to examine the influences of pearl farming practices on the benthic sediments and macrofauna of the surrounding marine environment of the Kimberley coast of Western Australia. Although, it was a so-called post-impact study utilising ACI design (*sensu* Glasby, 1997), we have attempted to redress some of the limitations of not having the "before" data by including greater spatial and temporal replication of both farms and reference sites. Furthermore we have included a Before/After, Control/Impact or "BACI" study that looks at the effect of *removing* a pearl farm on benthos. It was impossible to sample these pearl farms before they were established but we were able to investigate the effects of removing an established pearl farm and we continued monitoring for two years after its removal.

## INTRODUCTION

The gold or silver lipped pearl oyster, *Pinctada maxima,* forms the basis of Australia's pearl oyster culture industry located on the Kimberley coast of Western Australia (Prince 1999, Fletcher et al 2006). No artificial feed or chemicals are required in the culture of pearl oysters. The primary potential impact is thought to be the deposition of faeces and pseudofaeces from the cultured oysters and fouling organisms, and the fallout of debris during cleaning of the long lines that suspend the pearl oysters (Yokoyama 2002, Jernakoff 2002, O'Connor et al 2003, Gifford et al 2004).

Pearl oysters as other bivalves are filter feeders that feed on suspended particles from the water column. They then produce biodeposits in the form of faeces and pseudofaecal pellets as a waste product. It is thought that these biodeposits are similar in composition to the natural sediments because they are derived from phytoplankton and suspended particles (Grant et al 1995). However these biodeposits and shell debris could accumulate in the sediments below the oyster long lines and potentially lead to organic enrichment and even eutrophication (a detrimental increase of nutrients such as carbon and nitrogen). Further, the cleaning of biofouling organisms from oyster shells may accumulate beneath the lease. This may reduce oxygen content (Hatcher et al 1994), increase nutrient load and alter dependent benthic macrofaunal communities (Pearson and Rosenberg 1978, Kaspar et al 1985, Chamberlain et al 2001). Benthic macrofauna refer to the animals (greater than 0.5 or 1mm in size) that live or are associated with the sea floor and mostly consist of worms, molluscs including snails, crustacea (e.g. crabs and shrimps), echinoderms (seastars and brittlestars), fish, and other small animals.

The detection of aquaculture-related impacts in the marine environment, especially in the soft sediments of inshore regions, usually involves testing for nutrient and organic enrichment of the sediments and a change in benthic macrofaunal communities (e.g. Pearson and Rosenburg 1978, Grant et al 1995, Harstein and Rowden 2004). Benthic macrofauna are sensitive to organic enrichment levels perhaps undetectable via bulk chemical measures and can reflect an accumulation of impacts over time (Crawford et al 2003). Numerous studies on shellfish aquaculture have demonstrated that a change in benthic macrofaunal communities is one of the most sensitive measures of organic enrichment (Gibson et al 2000, Krassulya 2001, Dernie et al 2003, Thompson et al 2003, Barnes et al 2006).

In some parts of the world, mussel farms have been found to alter the characteristics of the seabed sediments (Grenz et al 1990). The mussel farms in sheltered sites have biodeposits and shell debris that can have build-up rates of 10cm/year which result in changes to the seabed approximately 20m from the farm boundaries (Dahlback and Gunnarsson 1981, Mattsson and Linden 1983). This build up of mussel biodeposits can create a situation of organic enrichment in the sediments under the farms (Grenz et al 1990). This can also cause an alteration of macrofaunal assemblages in these sediments (Mattsson and Linden 1983, Tenore et al 1985, Stenton- Dozey et al 1999, Mirto et al 2000, Christensen et al 2003, Giles et al 2006, Callier et al 2007).

This change in benthic macrofauna can include a decrease in the number of individuals and lower species richness (Mattsson and Lindén 1983, Kaspar et al 1985, Chamberlain et al 2001, Callier et al 2007). It might also involve a dominance of opportunistic species at mussel farms compared to reference sites (Chamberlain et al 2001: Site 2, Callier et al 2007) or the dominance of deposit feeders (Stenton- Dozey et al 1999).

However other studies have demonstrated that bivalve aquaculture may not cause a build up in sediment nutrients, nor cause a concurrent change in benthic macrofauna (Hatcher et al 1994, Grant et al 1995, Crawford et al 2003, Miron et al 2005, Goncalves da Costa and Cunha Nalesso 2006, Lasiak et al 2006). A study of cultured mussels at Twofold Bay, Eden NSW found there was no evidence of any ecological impact on the benthic macrofauna below the longlines (Lasiak et al 2006). The authors attributed their findings to the large, relatively open coastal area of Twofold Bay and suggested that mussel farms located in sheltered, poorly flushed areas where there is little opportunity for the dispersal of wastes away from culture sites can create nutrient enrichment and associated sediment changes (Lasiak et al 2006).

Other studies that have detected no significant impacts of mussel farms have also suggested that oceanographic characteristics are responsible for these findings (Chamberlain et al 2001, Hartstein and Rowden 2004, Miron et al 2005, Goncalves da Costa and Cunha Nalesso 2006). Chamberlain et al (2001) demonstrated that for mussel cultivation, the site with tidal flushing had negligibly impacted benthos, yet the benthos at a site with little tidal flushing had significant impacts. When currents are not strong enough to transport biodeposited material, the depth of the oxygenated layer of the sediment decreases and bottom oxygen may be depleted, leading to anoxia of the sediment and the overlying water (Chamberlain et al 2001).

In a study investigating the effects of different hydrodynamic regimes on biodeposits from mussel aquaculture, it was found that significant differences in macroinvertebrate assemblage composition occurred between farm and reference locations only at low energy sites (Hartstein and Rowden 2004). However, no such difference was observed between both farm and reference locations at the high-energy sites. The amount of total organic matter and mussel shell debris best explained the pattern of changes in the composition of

macroinvertebrate assemblages in the two low-energy study sites (Hartstein & Rowden, 2004). It was deduced that there is a relationship between hydrodynamic regime and subsequent modification of the macroinvertebrate assemblages possibly resulting from organic enrichment of seabed sediments by mussel biodeposits.

In general, the inconsistencies among studies may be attributed to differences in site hydrodynamics, topography, background enrichment, sediment type and especially culture characteristics such as bivalve stocking density, shell size and depth of line deployment (Callier et al 2007). For these reasons, the potential or predicted impacts of pearl oyster aquaculture cannot be assumed nor extrapolated from the numerous studies to date assessing effects of mussel aquaculture.

In terms of direct assessment of the potential impact of pearl aquaculture on marine benthos, comparatively fewer investigations have been undertaken to date. In 1998, a report commissioned by the *Pinctada maxima* pearling industry in WA (Enzer MEC 1998) suggested that the major environmental effect of pearl aquaculture in the region was the returning to the sea of material cleaned from pearl oyster shells. However, they suggested that because no chemicals are used in the cleaning process and the material returned is of marine origin, that the impact is temporally and spatially widely dispersed. In general, they found the industry to be environmentally benign. However this report did not directly test these assumptions with a sampling or monitoring regime.

Prince (1999) investigated the effects of *Pinctada maxima* aquaculture in the Montebellos Islands in WA and found no impact of the pearl farms on the abundance and diversity of the benthic macrofaunal community. There was, however, great variability in the fauna among individual sites, and control sites were not at comparable depths to lease sites. This study was also limited both spatially and temporally as it compared two sites within pearl farms to three nearby reference sites at only one period in time (March 1999).

An environmental audit and risk assessment of the pearl culture industry in Western Australia suggested that it would have a low environmental impact (Jernakoff 2002). However, they observed that there was scant scientific evidence to prove this point. The report recommended that a study should be undertaken to document whether this is in fact the case, and to quantify the extent to which pearling might change the natural environment (Jernakoff 2002). Four

environmental issues were recommended to be investigated:

1. The composition of the fouling growth cleaned from cultured shell;
2. The potential for modifying benthic habitat below pearl farms;
3. The disposal of grey water from vessels and shore camps; and
4. Monitoring interactions with protected fauna.

Another study examined the effects of a *Pinctada imbricata* pearl farm on sediment physico-chemical characteristics (total organic carbon, nitrogen and phosphorus) in Port Stephens, NSW (O'Connor et al 2003). This study was temporally replicated (n=6 sampling times), and compared five reference sites to one farm site. The sediment variables examined beneath the pearl lease did not differ significantly from the reference sites over the sampling times examined. Despite these findings, the authors acknowledged study limitations and called for future assessments of pearl aquaculture to incorporate benthic faunal community analyses and a Before/After, Control/Impact or "BACI" design whereby the sampling starts before the establishment of a farm.

Yokoyama (2006) compared the impacts of pearl farming and fish cages (yellowtail and seabream) in Gokasho Bay, Japan. The pearl farms in this region use rafts of *Pinctada martensii* (not longlines as in Australia) which covered 79000 $m^2$ of the bay and produced 800 kg of pearls in 1995 when this study was undertaken. They sampled the sediments under the pearl and fish farms and within reference sites for 18 months at monthly intervals. They compared the macrobenthic fauna as well as the sediment nutrient loads (carbon, nitrogen sulphide and dissolved oxygen) in these sites and found that fish farming created a large impact on the macrobenthic fauna and sediments, whereas pearl farming caused fewer effects. The community structure at the pearl farm site was similar to that at the control site, although there were lower densities and species diversity at the pearl farm site. There were also more seasonal changes in the dominant species at the pearl farm compared to the control sites.

Environment Australia approved a risk assessment of the Western Australia pearl oyster fishery in 2003 using the guidelines for the ecologically sustainable management (of fisheries) set out in the EPBC Act 1999. However this did not undertake any empirical monitoring or assessment of the potential impacts of the aquaculture operations. The Commonwealth Minister assessed the fishery as environmentally sustainable in 2003, but

required a reassessment after a 5 year period. In 2006, Fletcher *et al* compiled a comprehensive report on the contribution of the pearl oyster *Pinctada maxima* fishery to ecologically sustainable development (ESD) in Western Australia. This assessment examined the benefits and economic, social and environmental costs of the pearl oyster fishery but did not empirically test any potential environmental costs.

Collectively, few studies with sufficient temporal and spatial replication have been conducted to date to reliably assess the potential effects of pearl aquaculture operations on marine benthos. The current study represents the most comprehensive assessment of the effects of pearl aquaculture on benthic physico-chemistry and benthic macrofaunal communities undertaken internationally to date. The study represents a proactive collaboration between individual pearling operators, the Pearl Producers Association and scientists to redress this knowledge gap to ensure best practice environmental management of pearl leases of the Kimberley coast, North Western Australia.

**AIMS AND OBJECTIVES**

In this study we investigate the influence of pearl oyster *Pinctada maxima* culture on the benthic assemblages and sediment physico-chemistry of the Kimberley coast, Western Australia. We sampled the benthic macrofauna communities under the pearl long lines and compared these with communities from reference locations. We also measured the physico-chemistry of the sediments such as the redox potential, nutrients loads (nitrogen, carbon, phosphorus and carbonates) and total organic matter. These sediment variables were chosen because they have been identified as some of the most sensitive indicators of nutrient enrichment (Hargrave et al 1997).

We selected three pearl farms that have been in operation for between 10-40 years and are located in separate embayments or sounds around the Kimberley coast. The pearl oysters are suspended on panels (2-3m depth) that are held in place by floating long lines. The sediments under these long lines were sampled and compared to the sediments taken from four reference locations within the same embayment or area (total of 12 reference locations). We hypothesised that if the pearl farms are having an impact on the natural environment then we should detect differences in the sediments characteristics (physico-chemistry and benthic macrofauna) between the reference and farm locations.

We also determined if the closure of a pearl farm had an effect on the benthic sediments and associated benthic fauna. We selected a pearl farm that has been in operation for almost 50 years (Otama pearl farm, Kuri Bay) which was scheduled for closure. We sampled the sediments under the longlines before the farm was closed and for two sampling periods after closure (1 year and 2 years after). For each sampling period, we also sampled the sediments of three reference locations using a Before-After-Control-Impact (BACI) design (Underwood 1992). We hypothesised that if the farm was having an environmental impact on benthic conditions, then we would expect that the sediments under the farm to improve after removal of the farm, to match those of the reference locations. We would also expect the density and diversity of macrofauna to become more similar between the former farm and reference locations with time.

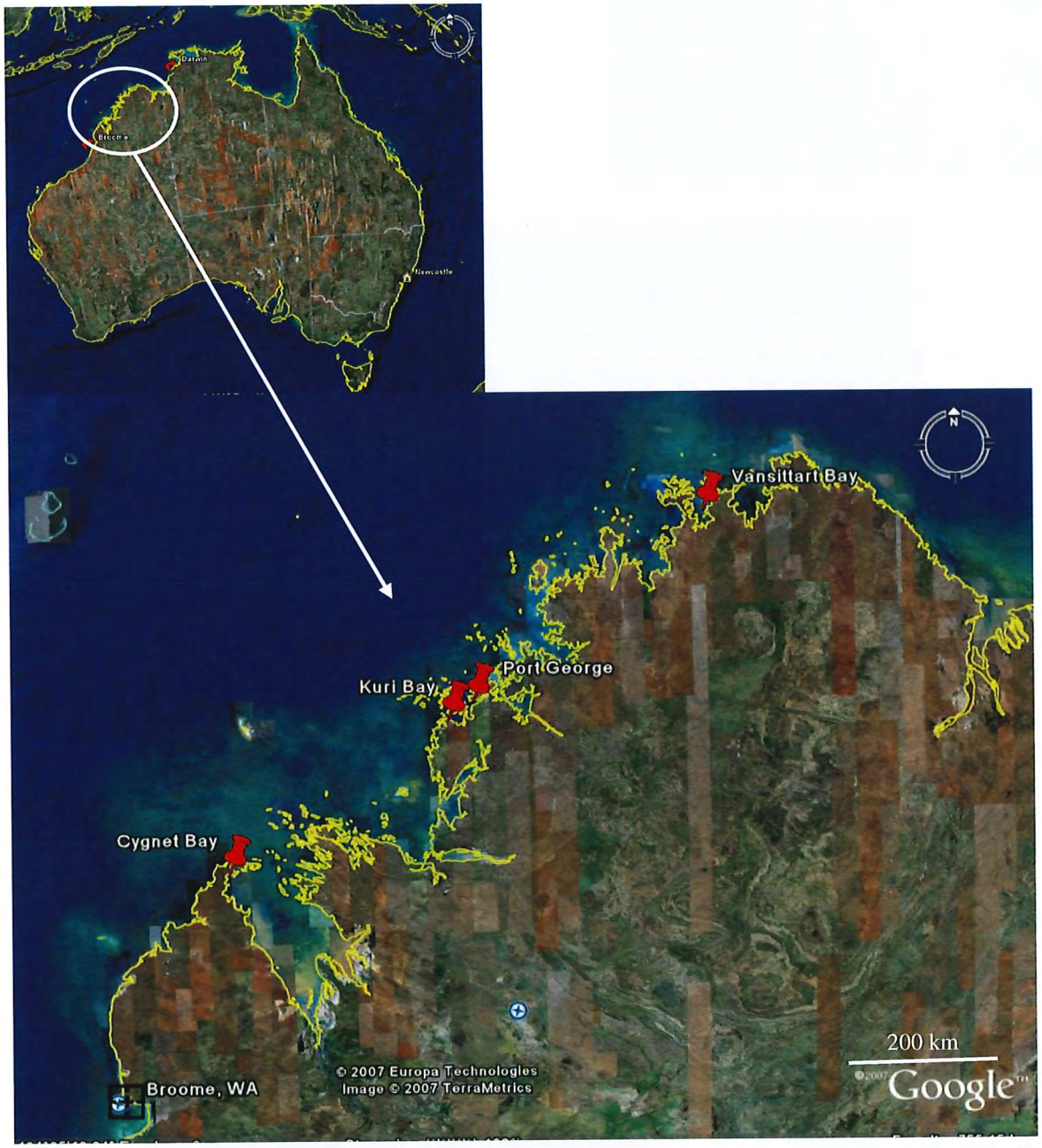


**Figure 1A:** Map of Australia showing the Kimberley region and the study areas. The main study was conducted in Cygnet, Port George and Vansittart Bays and the BACI study was conducted in Kuri Bay (All images taken from Google Earth © Europa Technologies http://earth.google.com/).

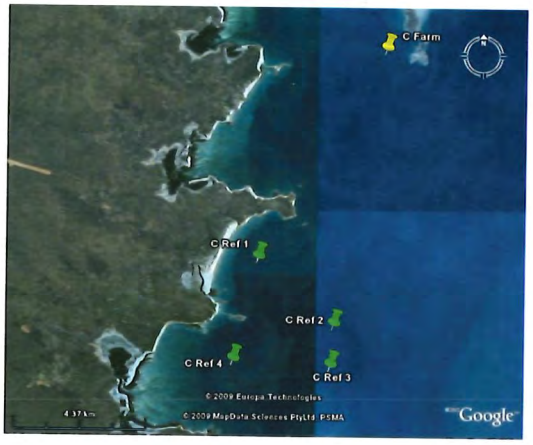


**Figure 1B**: Cygnet Bay (used for the main study) with reference (C Ref) and farm (C Farm) locations.


**Figure 1C**: Port George (used for the main study) with reference (P Ref) and farm (P Farm) locations.

**Figure 1D**: Vansittart Bay (used for the main study) with reference (V Ref) and farm (V Farm) locations.


**Figure 1E**: Kuri Bay (used for the BACI study) with reference (BACI Ref) and farm (BACI Farm) locations (All images from http://earth.google.com/)

## METHODS

### Study locations

The four pearl farms studied were located in the remote Kimberley coast of North Western Australia within the bays of Cygnet Bay (16°28'S, 123°02'E), Port George (15°23'S, 124°40'E) Kuri Bay (15°27'S, 124°31'E) and Vansittart Bay (14°01'S, 126°11'E) (Figures 1A-E). The pearl oysters were suspended (within the top 2-3m of water) on floating long lines (spaced over 50m apart). The industry standard stocking density is to have no more than 16250 shells per square nautical mile. Mussel farming (in New Zealand) suspends 800 mussels per m of long line (Hartstein and Rowden 2004), so by comparison to other shell aquacultures, pearl farming has low stocking densities.

The region has a tropical monsoon climate with annual maximum temperature of 32.1°C (minimum=23.1°C), annual rainfall between 766 to 1388 mm and 50-70% relative humidity (Bureau of Meteorology 2008). The wet season (December to March) may be subject to monsoons and even severe tropical cyclones (average rainfall between 167-310 mm/month) while the dry seasons (April to November) are more climatically stable and cooler (average rainfall between 10-14mm/month). The area is subject to diurnal (2 per day) tidal regimes with a maximum tidal range of 10.55m (mean spring range = 7.75m, mean neap range =5.72m). The area experiences strong bidirectional tidal velocities and as a result the water turbidity can be high. Turbidity measures in the region are recorded at 38,000 tonne of suspended sediment per tidal cycle or 35mg/L (courtesy of Paspaley Pearls). At Broome, the interactions between the Leeuwin current and Indonesian Flow Through (IFT) are distinct, with the ITF producing cooler waters and the Leeuwin current producing warmer waters. During sampling, the average surface temperature of the water was 28.85°C and surface salinity ranged from 30-35 psu. The sediment samples were taken from 10-16m water depth depending on the tidal state.

### Sampling design for the Main Study (comparison of existing farms with reference locations)

The main study investigated the pearl farms in three bays, Cygnet Bay, Port George and Vansittart Bay over 10 sampling occasions. The bays were separated from each other by 100's of kilometres. The sampling occurred over 2 years (October 2006 to November 2008). At each bay, the condition of the benthos within the pearl lease (farm) was compared to four

reference locations situated at least 1km from the pearl lease boundary (and 2-8 nautical miles from the farm), in similar water depths and sediment types. The design of this asymmetrical study compared the benthic conditions under each pearl farm (three in total) to four reference locations (twelve reference locations in total). At each of these locations, there were 3 study sites that were spaced 50 metres apart (similar to the spacing of the pearl farm long lines). Within each site, 3 grab and 3 core samples were collected; a total of 9 grabs and cores for each location, and 45 for each farm (Figure 2). The grab and core samples were collected ten times during the study (Grabs: Oct. 2006, Jan., May, Sept. and Nov.'07; Feb., April, May, Aug. and Nov. '08. Cores: Oct. 2006, Jan., Mar., May, Sept. and Nov.'07; Feb., April, May, and Aug. '08). There were some exceptions to this sampling regime as some samples were lost in transit and the omission of some redox readings on two farms due to the temporary malfunctions of the redox probe during the study. The September 2007 samples for Cygnet Bay and the redox readings for August and November 2008 for Vansittart and Port George are not included in the statistical analysis (see amended sampling times in ANOVA tables).

**Sampling design for BACI (Before, After Control, Impact) study (effect of farm closure)**

This study investigated the effects of removing a pearl farm (Otama pearl farm, near Kuri Bay) on the benthic conditions under the farm compared to nearby reference locations. This farm was de-commissioned in November 2006 and all adult shells were removed from the longlines (some juvenile shell remained for a few months). The design of this study included three sampling periods; before the pearl farm was closed down (1-6 months before), 6-12 months after removal of the shell and 18-24 months after removal. These three periods are referred to as "before", "one year after", and "two years after". Within each sampling period there were two sampling times (nested). This study was (referred to in the literature as a 'Beyond BACI' approach; Underwood, 1991, 1992) with the benthic conditions under one pearl farm compared to three reference locations located at least 1km from the pearl lease boundary (and 2-8 nautical miles from the farm), in similar water depths and sediment type. At each of these locations, there were 3 study sites that were spaced 50 metres apart (similar to the spacing of the pearl farm long lines). Within each site, 3 grab and 3 core samples were collected, which is a total of 9 grabs and cores for each location, and 36 in total per sampling time (Figure 3). The grab and cores samples occurred six times during the study: "before"- May and Oct. 2006, "one year after"- January and May '07, and "two years after"- May and Nov. '08.

**Sediment grabs for benthic macrofauna**

A Van Veen grab was used to collect the top layer of sediment (area = $0.1m^2$, depth=10cm), which was gently sieved through a 1mm mesh on site. The material retained on the sieve was preserved in 5% formalin-saline containing Rose Bengal that stained the fauna pink. The sample was then sieved again back at the laboratory and sorted for the macrofauna that were then preserved in 70% ethanol.

The benthic fauna were then identified to the lowest possible taxonomic level, usually genus or species although some fauna were identified only to order level. A low power dissecting microscope was used to count and identify the macrofauna. Among crustaceans, the most numerous group, decapods, were identified to the species or genus level. Amphipods, isopods and tanaids were identified from the species to family level and the ostracods, stomatopods, mysids, and cumaceans were identified to order level. The polychaetes (segmented worms) were identified to the family level while the molluscs (including the bivalves) were identified to genus or species level. The echinoderms were identified mostly to genus level; the fish were identified to family level and the few sea spiders collected were identified to genus level. A small percentage of the benthic fauna included worms (flat, ribbon and acorn worms) sipunculids, sponges, cnidarians and large foraminifera's were not identified, however they were counted.

**Sediment cores for physico-chemical analysis**

A universal gravity corer (68mm in diameter, Aquatic Research Instruments, Idaho USA) was used to collect the sediments for physico-chemical analysis. The corer collected over 20cm of sediment but only the top 5cm of sediment was used. The redox potential of the sediment was measured immediately after collection using a handheld pH-mV-Temp. meter (TPS Pty Ltd, Brisbane, Australia). Organic enrichment of marine benthic habitats usually involves an increased demand of sediment oxygen by benthic micro-organisms and fauna, and this subsequently depletes the sediment oxygen content (Pearson and Rosenberg 1978). A probe and meter that measures the Redox potential profile can detect this depletion of sediment oxygen and this measure was used as a surrogate of organic enrichment (as used by Grant et al 1995, Chamberlain et al 2001). In our study, the pH of the sediment was concurrently measured using pH indictor sticks. The sample was then placed into a sealed container, frozen and transported back to the laboratory where they were oven dried at 40°C

and then ground. The physico-chemistry variables measured in the laboratory from the sediment core samples were total organic matter, carbon, nitrogen, phosphorus, and carbonates.

The total organic matter was measured using the loss on ignition (LOI), furnace method (400°C, 16 hours) (Heiri et al 2001). The total nitrogen and carbon content was measured using a LECO *Tru-spec*® CNS induction furnace analyser. Total phosphorus was determined photometrically after converting the organic phosphorus to inorganic phosphorus (furnace method: 550°C, 2 hours). The sample was then analysed using the ascorbic acid method for phosphorus analysis (Kuo 1996). The carbonates were measured using the sequential loss on ignition method (Dean 1974).

**Univariate statistical analysis for the Main Study (comparison of existing farms with reference locations)**

The primary main aim of this study, with the broadest implications, was to compare three farm locations with 12 reference locations so an asymmetrical analysis of variance (ANOVA) was used. This design tests the more global question of whether pearl farms, in the general Kimberly region, have an impact on underlying benthic conditions compared to reference locations. This was a four-factor mixed model ANOVA design that could compare the sediments and benthic fauna across all sites, locations, for all bays, across all sampling times. The first factor was time (random factor); the second factor was bay (fixed factor); the third factor was location ($n$=12 reference locations and $n$=3 farm locations, random and nested in bay); and the fourth factor was sites (nested within location and bays). The variables compared were the number of benthic species/families and individuals per grab and the sediment redox potential, total organic matter, total nitrogen, total phosphorus, total carbon and carbonates.

A second analysis was performed on the data associated with the asymmetrical component; i.e. comparing farm and reference locations. This was done by comparing the variance among all locations (farms and references) with the variance among just the reference locations (calculated by subtracting the sum squares of the second analysis from the first). The remaining variance can be attributed to any difference attributable to the farm locations (i.e. a measure of their potential impact). The design is shown in Figure 2 and this calculation was performed for the main factors; location (nested in bays), sites (nested in location and bays)

and the interaction term location (bay) x time. Appropriate *F* tests were constructed according to Underwood 1981. This type of asymmetrical ANOVA calculation has been employed by others when undertaking environmental impact assessment and ecological studies (e.g. Glasby 1997, Roberts et al 1998, O'Connor et al 2003, Terlizzi et al 2005, Lasiak et al 2006, Gladstone 2007).

Another batch of asymmetrical ANOVAs was performed as above but separately for each bay. This second series of ANOVAs tested whether an individual farm had an impact within a particular bay, and allowed explicit assessment of an individual farm. This was a three factor mixed model ANOVA and the first factor was time (random factor); the second factor was location (comparing four reference locations with one farm location, random); and the third factor was sites (nested within location). As above, a second analysis was performed on the data associated with the asymmetrical component; i.e. comparing farm and reference locations. This three-factor ANOVA allowed for comparisons within a bay and the same variables were tested.

**Univariate statistical analysis for the BACI study (effect of farm closure)**

An asymmetrical analysis of variance (ANOVA) was used to compare the sediments (and associated benthic fauna) over three sampling periods; before a pearl farm was closed down, soon after closing (within a year) and later (between 18-24 months later). A four-factor mixed model ANOVA was used to compare the sediments and benthic fauna across all sites, locations, for two sampling times within these three periods (which we will call "Before", "After 1" and "After 2"). The first factor was period (Before, After 1 and After 2), the second factor was time ($n$=2, nested in period and random); the third factor was location ($n$=4, orthogonal and random, three reference locations and one farm location); and the fourth factor was sites (nested within location). The variables compared were the number of benthic taxa and individuals per grab and the sediment total organic matter, total nitrogen, total carbon and carbonates.

As previously described for the main study, a second analysis was performed on only the data associated with the asymmetrical component; i.e. comparing farm and reference locations. The design is shown in Figure 2 and this calculation was performed for the main factors; location, sites (nested in location) and the interaction terms; period x location, period x sites (location), location x time (period) and sites (location) x time (period). Only those tests

relevant to the main question (i.e. farm vs. reference locations) will be discussed within the results.

For all ANOVA tests, a Cochran's test was used to detect heterogeneity of variances and the data were transformed (Ln (x+1)) if the Cochran's test was significant (Winer 1971). In some cases where homogeneity of variance could not be achieved the ANOVA results were interpreted with caution. When a significant interaction term occurred, we performed 2-way asymmetrical ANOVAs for each time; the first factor was location (comparing four reference locations with one farm location, random); and the second factor was sites (nested within location). This was used as a post-hoc test to determine the pattern of the differences detected.

**Multivariate statistical analysis for Main Study (comparison of existing farms with reference locations)**

To compare the composition and abundance of the benthic assemblages between the farm and reference locations, multivariate statistical analyses were performed for each sampling time (using the PRIMER package, Clark and Warwick 2001). For all comparisons, a Bray-Curtis similarity analysis between samples was performed after a square root transformation and used to create a non-metric multidimensional scaling (nMDS) plot. The replicates at each site were pooled to make the MDS plots clearer to read. A 2-way nested ANOSIM (analysis of similarity) was used to compare the assemblages among the three bays (averaged across all farm and reference groups) and then between farm and reference locations (averaged across all bay groups).

**Multivariate statistical analysis for BACI study (effect of farm closure)**

To compare the composition and abundance of the benthic assemblages between the farm and reference locations, before and after farm removal, multivariate statistical analyses were performed for each sampling time (using the PRIMER package, Clark and Warwick 2001). This was similar to the tests as described above for the main study however the 2-way nested ANOSIM (analysis of similarity) was used to compare the assemblages among the three sampling periods (averaged across all farm and reference groups) and then between farm and reference locations (averaged across all sampling periods).

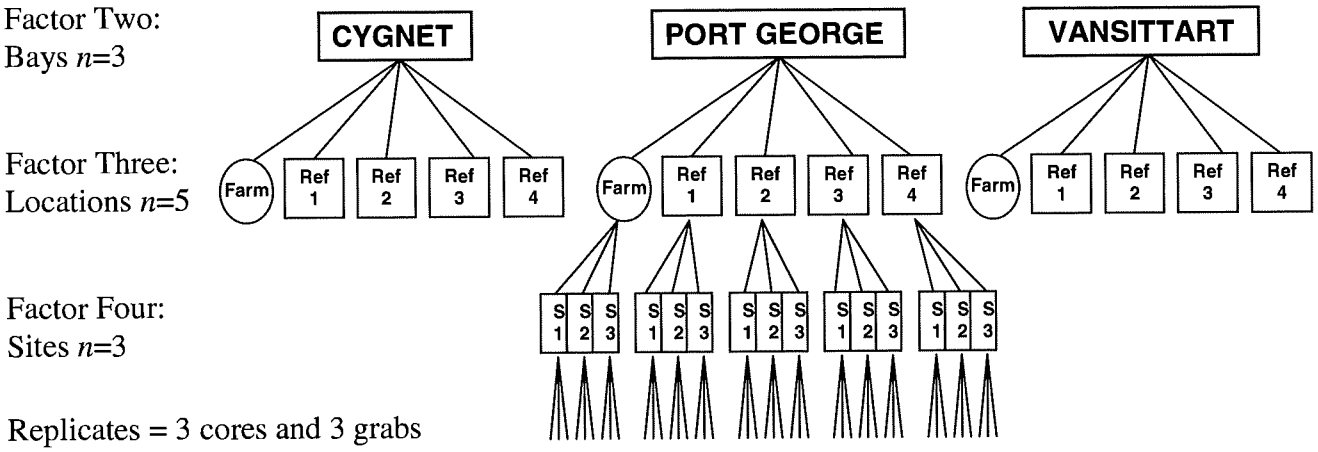


**Figure 2.** The design of the Main Study. There were ten sampling times (not shown) in three bays, each of which contained five (nested) locations (one farm and four references). Each location contained three (nested) sites (S) at which 3 cores and 3 grabs were taken for each time.

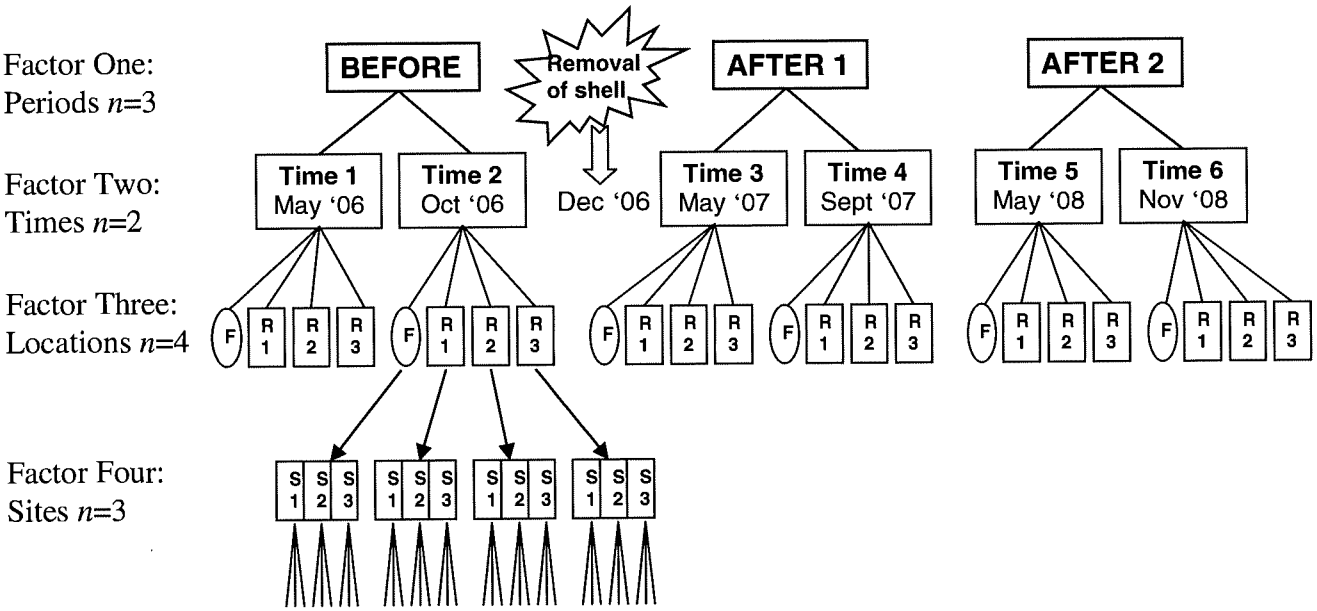


**Figure 3.** The design of the Beyond BACI (Before, After, Control, Impact) study in Kuri Bay. There were 3 sampling periods; before the pearl farm was closed down (Before), 6-12 months after (After 1) and 18-24 months after removal (After 2). Within each period there were two (nested) sampling times and four locations sampled (one farm and three references). At each location there were 3 (nested) sites at which 3 cores and 3 grabs were taken for each time.

## RESULTS

### Main Study (comparison of existing farms with reference locations)

We collected 242 benthic macrofauna taxa (22,015 individuals) over a two year sampling period (October 2006 to November 2008) representing 16 Phyla and 156 families (Appendix 1, Table 1). The fauna were typical of the tropical Indo-Pacific found in northern regions of Australia (Clark and Rowe 1971, Lamprell and Whitehead 1992, Lamprell and Healy 1998, Poore 2003, Wilson et al 2003, Marsh-Morris 2004, Bamber 2005). Numerically the taxa were dominated by Polychaeta (35%), Crustacea (28%), Mollusca (20%), and Echinodermata (13%). Other groups such as Sipunculids, Cnidarians and fish represented 4% of the individuals. The samples were dominated numerically by the Brittle star Amphuridae *Lymanella laevis* ($n$=2735), Tanaids ($n$=2426), followed by the polychaetes Trichobranchidae ($n$=936) and Terebellidae ($n$=865), and the bivalve *Theora fragilis* ($n$=846) (Appendix 1, Table 1A-E). In addition, new species were potentially found, including a new genus of polychaete (Family Flabigelleridae) although the taxonomic classification of this fauna is yet to be confirmed.

### Main study: Univariate Results

For all the ANOVAs run comparing all three bays we found a significant difference among sites (nested in locations) which suggests the variability among sites was great. These analyses also demonstrated that the sites were not consistently different over time; i.e. the variability among sites changed over time.

Main Study: The number of macrobenthic taxa

The mean number of taxa per grab ranged from 1.22 at one location at one time to as high as 15.8 at another with an overall mean of $7.87^{\pm}1.08$ (Figure 4). The number of macrobenthic taxa per $0.1m^2$ of soft sediments under the farm longlines was not consistently different to that found in the references locations across all three bays pooled over all sampling times (Table A2.1. Loc(Bay); Farms vs. Refs $F$=0.303, $P$=0.822). There was also no difference in the pattern of variability between all farms and reference locations (pooled across bays) among the different sampling times (Table A2.1. Loc(Bay) x Time; Farms vs. Refs $F$=1.05, $P$=0.418). Individual bay analysis also demonstrated that at no time in any bay was there a difference between farm and reference locations (Tables A3.1, A3.9, A3.17).

Main Study: The number of macrobenthic individuals

The average number of macroinvertebrate individuals per grab was 14.79±2.66, with Cygnet Bay (mean= 17.73± 2.95) and Vansittart Bay (mean= 17.74± 3.45) containing more individuals than Port George (mean= 13.40± 2.35) (Figure 5). Similar to the findings for the number of taxa, the numbers of macrobenthic individuals underlying all farms were not consistently different to that found on average among the references across all three bays (Figure 5, Table A2.2. Loc(Bay); Farms vs. Refs $F$=1.20, $P$=0.364) and this pattern did not change over time (Table A2.2. Loc(Bay) x Time: Farms vs. Refs $F$=0.878, $P$=0.639). Further, individual bay analysis also demonstrated that at no time, in any one bay, was there a difference between a farm and reference locations (Tables A3.2, A3.10, A3.18). In Cygnet Bay during October 2006 the number of individuals was somewhat higher at the farm compared to the reference locations (Figure 5); however, this was not a significant difference and was not sustained over time (Table A3.18).

Main Study: Total Organic Matter (%TOM) in sediments

The %TOM varied between bays with Vansittart (mean= 9.91±0.30 %TOM) having on average greater amounts of %TOM than Port George (mean= 6.60± 0.27) and Cygnet Bay (mean= 4.97±0.12) (Figure 6). Overall, the %TOM in sediments under all farm longlines was not consistently different to the variability of %TOM found at all the reference locations, nor were there any such differences between farms and references at any particular point in time (Figure 6, Table A2.3. Loc(Bay); Farms vs. Refs $F$=5.29, $P$=0.673, Loc(Bay) x Time; Farms vs. Refs, $F$=0.966, $P$=0.519). Individual bay analysis also demonstrated that at no time, in any bay, was there a difference between a farm and reference locations (Tables A3.3, A3.11, A3.19).

In Cygnet Bay there was a change in the pattern of variability at the sites nested within the farm locations compared to the fluctuations at the reference locations (Table A3.3). In Port George there was greater site variability at the farm compared to the reference locations and this pattern changed over time (Table A3.11). However neither of these contributed to an overall difference between farm and references at the scale of locations for %TOM.

Main Study: Nitrogen content (%N) of sediments

The %N content in sediments was different among the bays in a similar pattern to the %TOM content (i.e. Vansittart > Port George > Cygnet Bay, $0.147^{\pm}0.011 > 0.078^{\pm}0.008 > 0.056^{\pm}0.006$ %N respectively) (Figure 7). Across all bays, there was no consistent difference in the variability of %N in soft sediments between all farms and reference locations, across all times, nor at any single sampling interval (Figure 7, Table A2.4. Loc(Bay) x Time; Farms vs. Refs $F$=0.608, $P$=0.914; Farms vs. Refs $F$=0.978, $P$=0.445).

In Port George however, the farm had significantly higher %N content than the reference locations within this bay (Figure 7, Table A3.12. Loc Farm vs. Refs. $F$=16.71, $P$ =0.026). However, this was not observed at the other farms (Tables A3.4 and A3.20) excepting one occasion at Vansittart Bay (Table A3.20. Loc(Bay) x Time; Farms vs. Refs $F$=2.55, $P$=0.029). In September 2007, the sediments under the farm at Vansittart, were significantly greater in %N than the reference locations (2-way ANOVA $F$=5.19, $P$=0.046). The elevated %N values found at the farm in Port George however, were within the range of values observed at the reference locations in other bays.

Main Study: Carbon content (%C) of sediments

The %C content in sediments was different among the bays with Cygnet Bay having greater percentages (mean $=6.43^{\pm}0.17$ %C) than Port George (mean $=6.85^{\pm}0.12$) and Vansittart (mean=$5.00^{\pm}0.20$) (Figure 8). This is the opposite of the pattern observed for %TOM and %N (Figures 6 & 7). There was no consistent difference in %C variation under all farm longlines to that found on average among references locations across all three bays pooled over all sampling times, nor were there any differences between farms compared to references at any one time (Figure 8, Table A2.5. Loc(Bay); Farms vs. Refs $F$=0.881, $P$=0.487; Loc(Bay) x Time; Farms vs. Refs $F$=1.02, $P$=0.454). Similarly, individual bay analyses also demonstrated that at no time, in any bay, was there a difference between the farm and reference locations (Tables A3.5, A3.13, A3.21).

Main Study: Phosphorus content (P µg/g) of sediments

The P µg/g content of sediments varied between bays in a similar pattern to %TOM and %N (Vansittart > Port George > Cygnet Bay, means = $618^{\pm}17$, $624^{\pm}17$ and $485^{\pm}25$ P µg/g respectively) (Figure 9). There were no consistent overall differences in P µg/g content between farms and reference locations across the three bays, nor were there any significant

differences between farms and reference locations at any point in time (Table A2.6. Loc(Bay) x Time: Farms vs. Refs $F$=1.090, $P$=0.381; Loc(Bay); Farms vs. Refs $F$=0.651, $P$=0.602). Individual bay analysis also demonstrated that there was no difference in P µg/g content between the farm and reference locations in any one bay at any time (Tables A3.6, A3.14 and A3.22).

Main Study: Carbonate content (%$CO_3$) of sediments

A similar pattern was observed for % $CO_3$ as observed for sediment %C (Cygnet Bay > Port George > Vansittart, means = 21.08$^{\pm}$0.52, 19.57$^{\pm}$0.38 and 9.87$^{\pm}$0.89 % $CO_3$ respectively) (Figure 10). The %$CO_3$ found in the soft sediments under the farm longlines was not different to that found in the references locations (Figure 10, Table A2.7. Loc(Bay); Farms vs. Refs $F$=0.511, $P$=0.685). Nor were there differences between farms and reference locations at any particular sampling time (Table A2.7. Loc(Bay) x Time; Farms vs. Refs $F$=0.567, $P$=0.940). Individual bay analysis also demonstrated that at no time, in any of the bays, was there a difference between a farm and reference locations (Tables A3.7, A3.15 and A3.23).

Main Study: Redox potential (mV) of sediments

The range of redox potential (mV) in the sediments did not vary greatly between bays (means: Vansittart = -281$^{\pm}$16, Port George = -253$^{\pm}$12, Cygnet Bay = -251$^{\pm}$17mV) (Figure 11). There was no difference in the variability among all farms compared to all reference locations for the redox potential of the soft sediments, nor at any particular sampling time were differences observed (Figure 11, Table A2.8. Loc(Bay); Farms vs. Refs $F$=1.37, $P$=0.314; Loc(Bay) x Time: Farms vs. Refs $F$=0.437, $P$=0.972). At no time in any bay was there a difference in the redox potential between the farm and reference locations (Tables A3.8, A3.16 and A3.24).

Main Study: Multivariate analysis of benthic macrofauna assemblages

The benthic assemblages in the soft sediments were compared between farm and reference locations across all three bays (Cygnet, Port George and Vansittart). The multivariate analysis has revealed that there were no consistent differences in the benthic assemblages under the longlines (farm locations) when compared to the reference locations (Figures 12 & 13) for all time periods. An analysis of similarity (ANOSIM) test for each sampling time revealed that the bays had different benthic fauna assemblages from one another, as would be expected of different geographical regions (Table 1). However, the benthic assemblage under

each farm location was similar to the reference locations located within the region (bay). The farms did not create a unique or distinct benthic assemblage from the reference locations (Table 1).

**Table 1:** Results of the 2-way nested ANOSIM (Analysis of Similarity) test for detecting differences between bays (averaged across all farms vs. refs locations); and between farm versus reference locations (using bays groups as samples). For the bay comparisons there were 999 permutations used (random samples). For the farm versus reference comparison there were only 10 permutations used for each time.

| | **Bays (Cygnet vs. Port George vs. Vansittart Bays)** | | **Farms vs. Reference locations (nested within Bays)** | |
|---|---|---|---|---|
| **Sampling time** | **Global R** | ***P*** | **Global R** | ***P*** |
| **Oct. 06** | 0.479 | 0.001 | -0.963 | 1 |
| **Jan. 07** | 0.427 | 0.001 | -0.593 | 1 |
| **May 07** | 0.400 | 0.001 | -0.704 | 1 |
| **Sept. 07** | 0.502 | 0.001 | -0.704 | 1 |
| **Nov. 07** | 0.480 | 0.001 | -0.556 | 1 |
| **Feb. 08** | 0.377 | 0.001 | -0.593 | 1 |
| **April 08** | 0.482 | 0.001 | -0.593 | 1 |
| **May 08** | 0.371 | 0.001 | -0.778 | 1 |
| **Aug. 08** | 0.340 | 0.001 | -0.667 | 1 |
| **Nov. 08** | 0.479 | 0.001 | -0.741 | 1 |

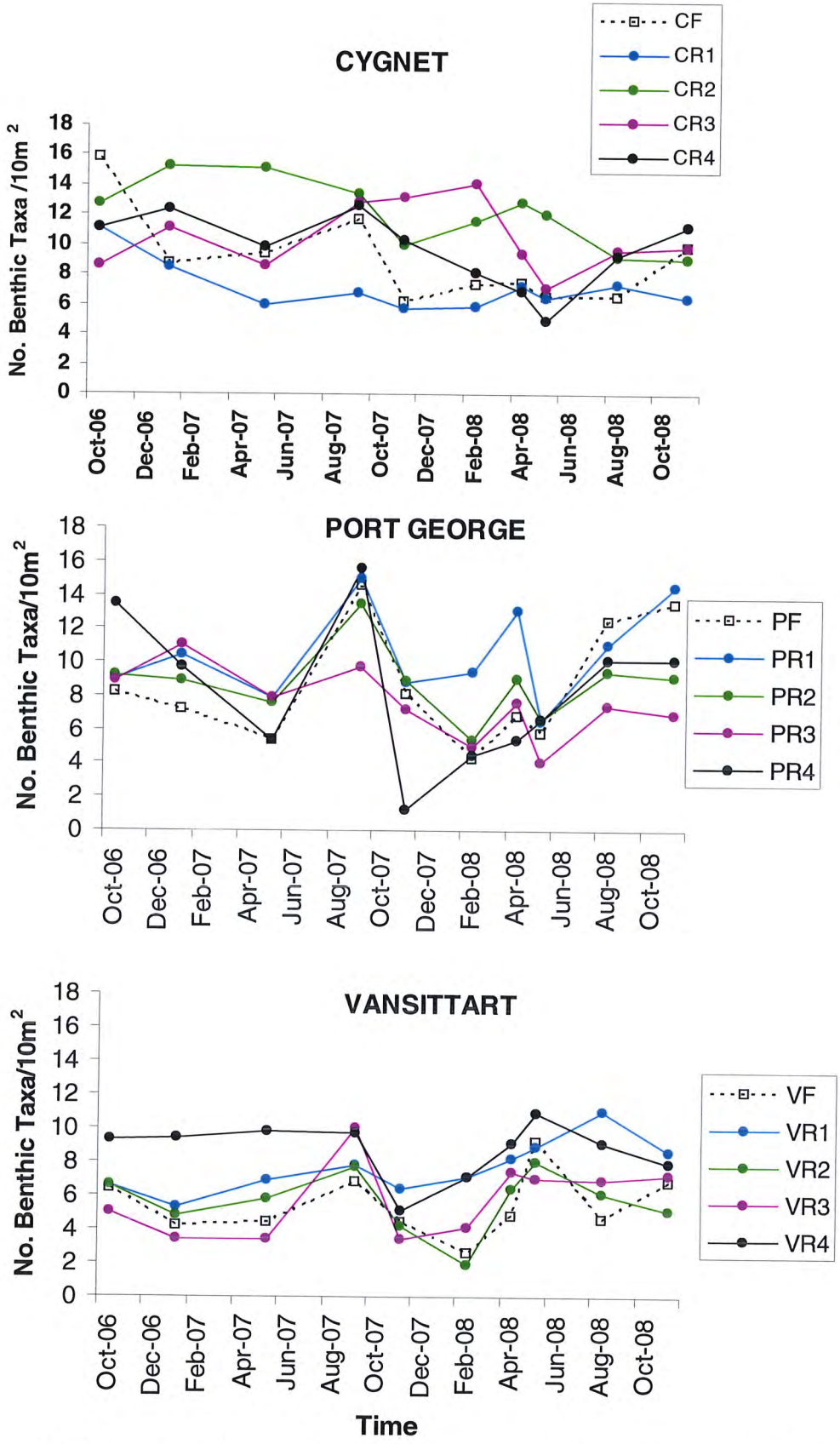


Figure 4. The number of macrobenthic taxa (species to families) per 0.1m$^2$ of soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

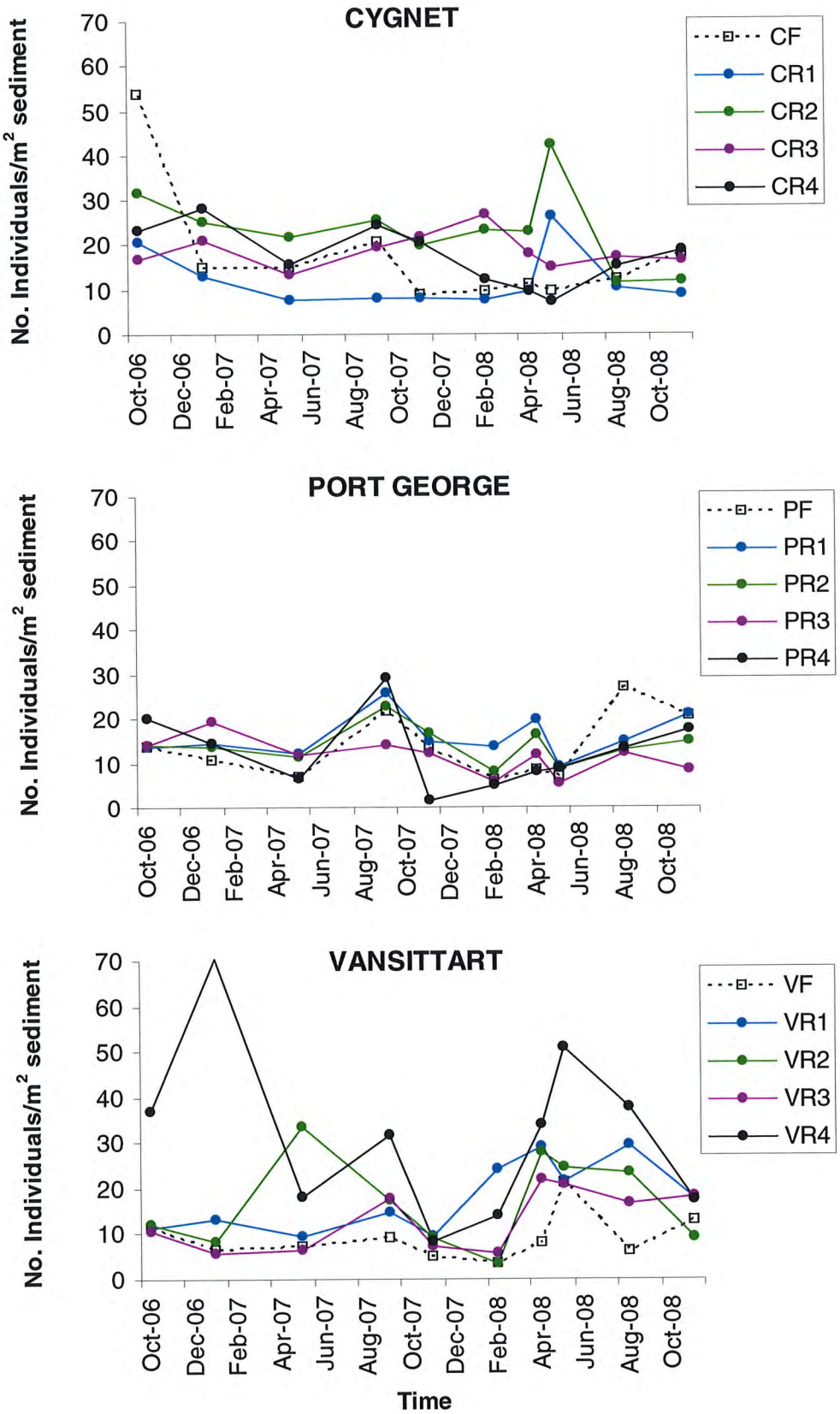


Figure 5. The number of macrobenthic invertebrates per $0.1m^2$ of soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

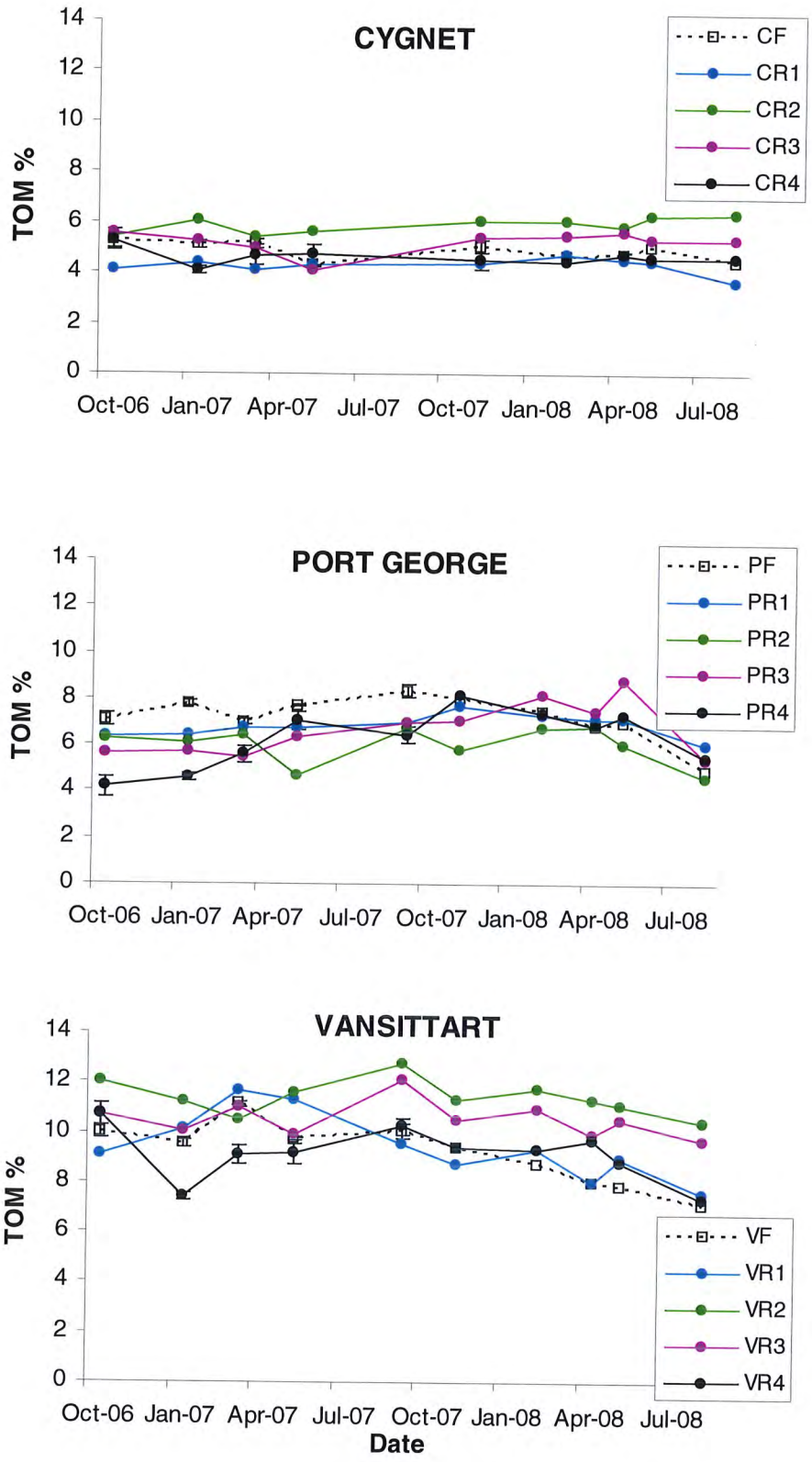


Figure 6. The percentage of Total Organic Matter in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

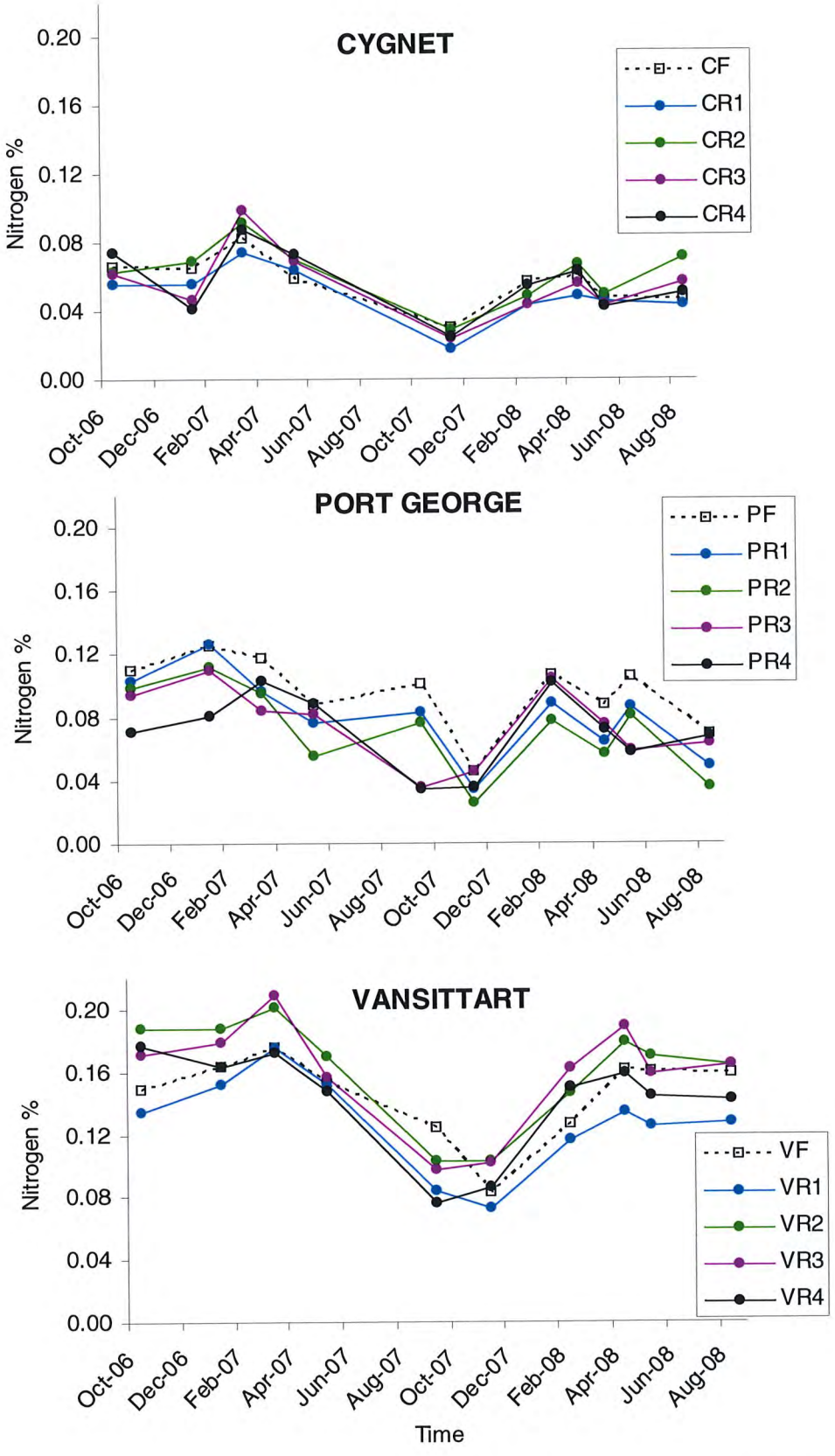


Figure 7. The percentage of Nitrogen in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

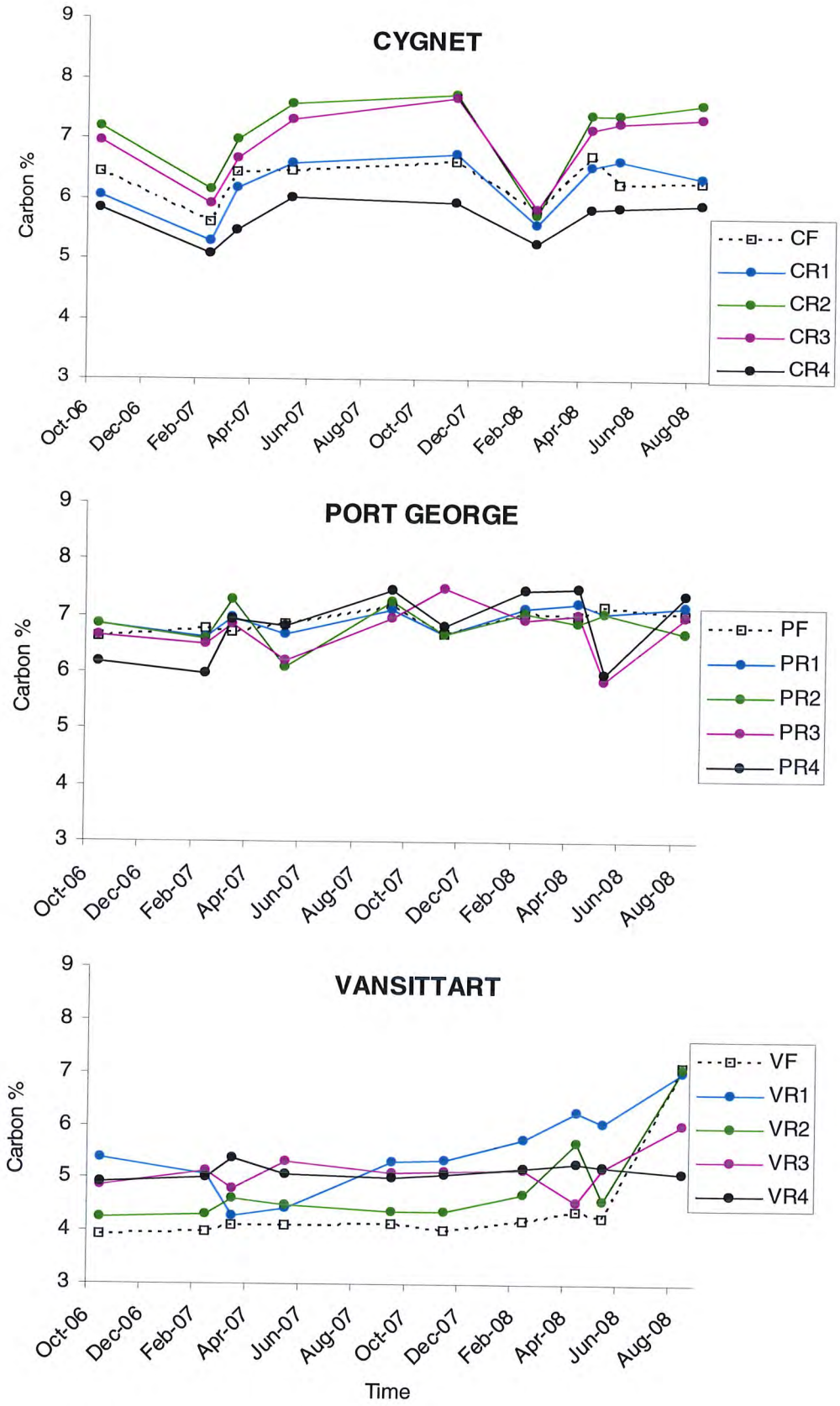


Figure 8. The percentage of Carbon in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

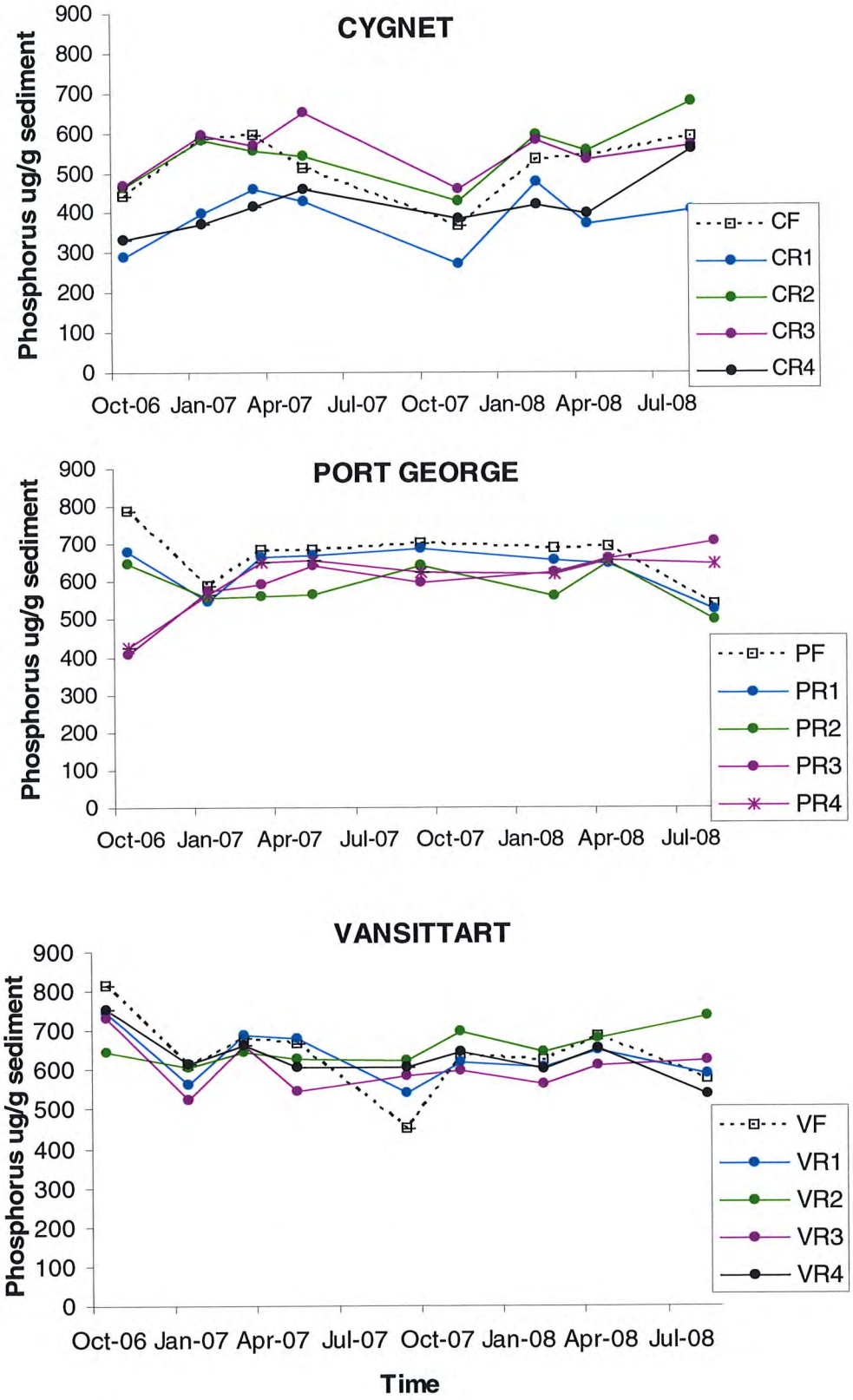


Figure 9. The phosphorus content (µg/g) in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

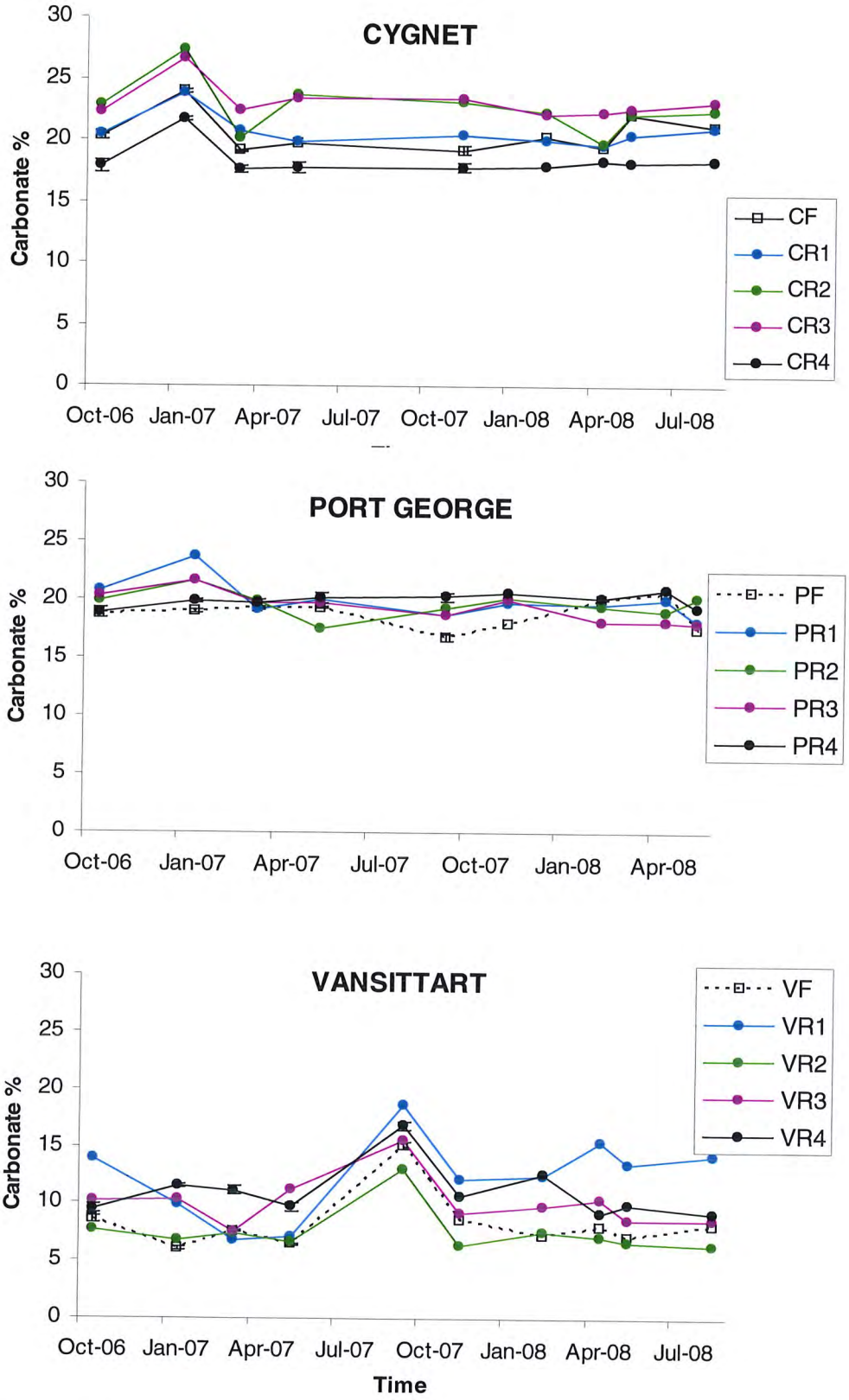


Figure 10. The percentage of carbonates in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

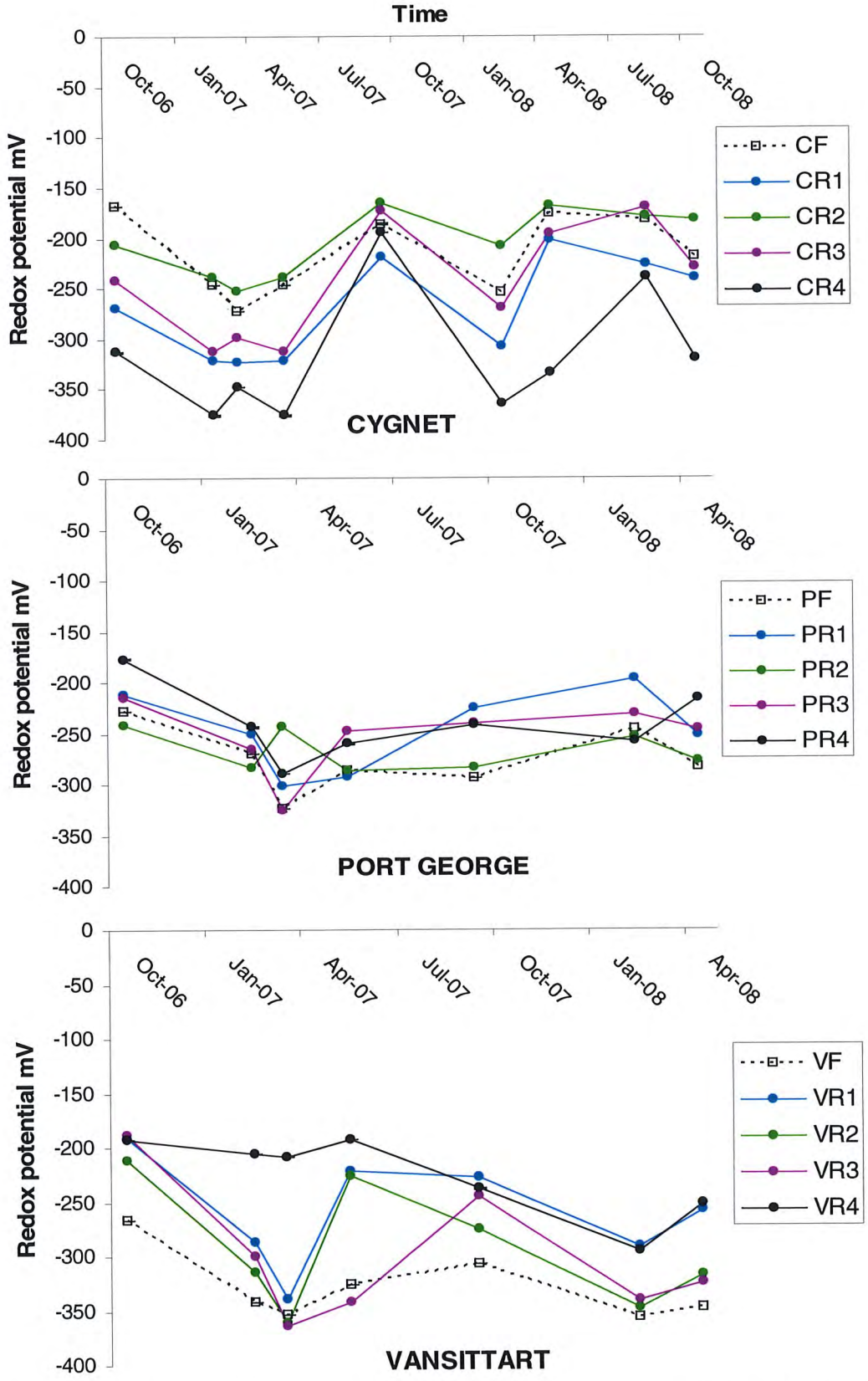


Figure 11. The redox potential (mV) in the soft sediments at each bay (Cygnet, Port George and Vansittart) over 2 years sampling among farm (F) and references locations (R) in the main study.

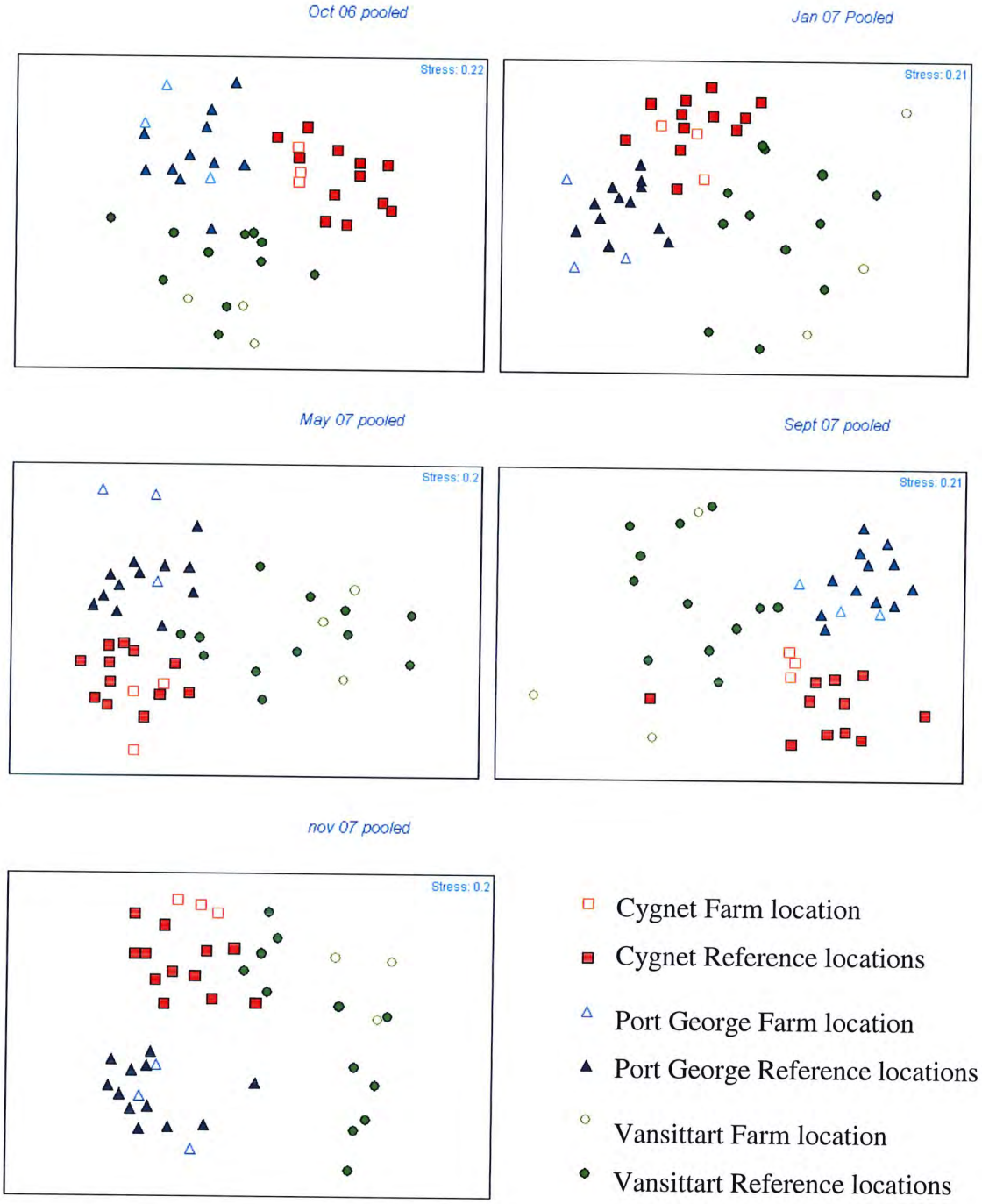


Figure 12. The MDS (multi-dimensional scaling) plots representing the assemblages of macrobenthic fauna within the bays (Cygnet, Port George and Vansittart), between farm and reference locations, for five sampling times (October 2006, January, May, September and November 2007).

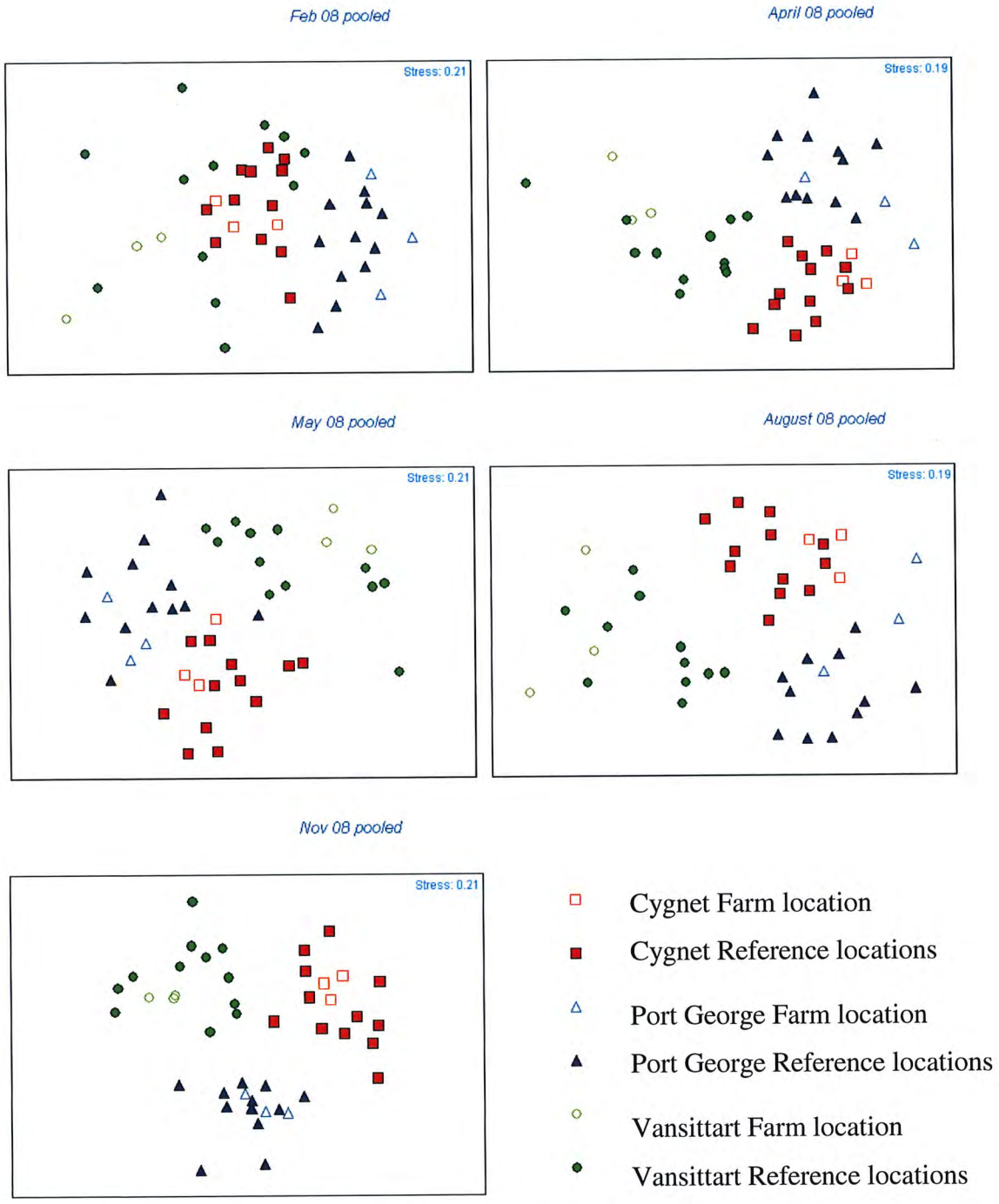


Figure 13. The MDS (multi-dimensional scaling) plots representing the assemblages of macrobenthic fauna within the bays (Cygnet, Port George and Vansittart), between farm and reference locations, for five sampling times (January, April, May, August and November 2008).

**Results of BACI Study (effect of farm closure)**

**BACI Univariate Results**

We collected 85 benthic macrofauna taxa (2,880 individuals) over a three year sampling period (May 2006 to November 2008) (Appendix 1, Table A2). The removal of the pearl oyster shell from the longlines did not create a significant change in the numbers of macrofauna (number of taxa and individuals) at the farm compared to the variability of macrofauna at the reference locations (Figures 14 & 15). Similarly, removal of the pearl oyster shell did not cause significant changes for most physicochemical variables among farm and reference locations. For each ANOVA (every variable tested) there was significant difference in the variability of the sites (nested in location) over time (Tables A4.1-A4.6; Time (Per) x Site (Loc) interaction term).

BACI study: The number of macrobenthic taxa

There was an average of $8.13^{\pm}0.6$ taxa collected per grab in this study although this ranged over time from $4.86^{\pm}0.4$ (in May '08) to $11.83^{\pm}0.7$ taxa (in Sept. '07) (Figure 14a). Overall the variability in the number of macrobenthic taxa was similar among the pearl farm and reference locations, before and up to two years after farm removal (Table A4.1. Period x Location; Farm vs. Refs $F$=0.18, $P$=0.844). There was also no change in the pattern of variation between the farm and reference locations among times within a period (Table A4.1 Time (Per) x Loc; Farm vs. Refs $F$=0.93, $P$=0.481).

BACI study: The number of macrobenthic individuals

There was an overall average of $8.13^{\pm}0.6$ macrobenthic individuals collected per grab, with May '08 having the lowest abundances (mean=$6.25^{\pm}0.5$) and May '06 having the greatest (mean=$17.25^{\pm}3.5$) (Figure 14b). The variability in abundance of macrobenthic individuals at the pearl farm was consistently within the range of variability found among reference locations, before and up to two years after farm removal (Table A4.2 Period x Location; Farm vs. Refs $F$=5.61, $P$=0.069). There was also no change in the pattern of variation between the farm and reference locations among times within a period (Table A4.2 Time (Per) x Loc; Farm vs. Refs $F$=0.59, $P$=0.644).

BACI study: The Total Organic Matter (%TOM) content of sediments

The sediments of all locations had an average of $6.38^{\pm}0.14$ %TOM over all times and ranged from $3.77^{\pm}0.15$ in Nov. '08 to $8.32^{\pm}0.14$ %TOM in Oct. '06 (Figure 15a). The %TOM in the

sediments underlying the pearl farm was similar to the variability of %TOM found in sediments among reference locations, before and up to two years after farm removal (Figure 15a, Table A4.3 Period x Location; Farm vs. Refs $F$=0.069, $P$=0.934). There was also no change in the pattern of variation between the farm and reference locations among times within a period (Table A4.3 Time (Per) x Loc; Farm vs. Refs $F$=2.12, $P$=0.199).

BACI study: The Nitrogen content (%N) of sediments

There was little variation in the %N content of the sediments over time (overall mean = 0.0704$^{\pm}$0.002 %N) (Figure 15b). The changes in the %N at the farms were generally in the range observed among reference locations, before and up to two years after farm removal (Figure 15b, Table A4.4 Period x Location; Farm vs. Refs $F$=2.74, $P$=0.178). However, during one time period (before the removal of the farm) the pearl farm was different compared to the reference locations (Table A4.4 Time (Per) x Loc; Farm vs. Refs $F$=5.00, $P$=0.045). The difference can be attributed to the farm having lower nitrogen during October 2006 (2-way ANOVA; $F$=15.5, $P$=0.029). This difference was not consistent, however, when compared to the earlier sampling event (May 2006) prior to farm removal.

BACI study: The Carbon content (%C) of sediments

There was little variation in the %C observed over time in this study (overall mean = 6.72$^{\pm}$0.09 %C) (Figure 16a). The variability of %C in the sediments under the pearl farm was consistently within the range found among reference locations, before and up to two years after farm removal (Figure 16a, Table A4.5 Period x Location; Farm vs. Refs $F$=3.85, $P$=0.117). There was also no change in this pattern between the farm and reference locations among times within a period (Table A4.5 Time (Per) x Loc; Farm vs. Refs $F$=4.35, $P$=0.060).

BACI study: The Carbonates content (%$CO_3$) of sediments

Similar patterns were observed for the % $CO_3$ in the sediments over time in this study (overall mean = 19.90$^{\pm}$0.34 %$CO_3$) (Figure 16b). The %$CO_3$ in the sediments under the pearl farm was within the variability found among reference locations, before and up to two years after farm removal (Figure 16b, Table A4.6 Period x Location; Farm vs. Refs $F$=5.61, $P$=0.069). There was also no change in the pattern of variation between the farm and

reference locations over time (Table A4.6 Time (Per) x Loc; Farm vs. Refs $F$=3.76, $P$=0.079) although there was greater site variability at the farm compared to the reference locations (Table A4.6. Sites (farm), $F$=15.25, $P$=0.008) which changed over time (Table A4.6. Time (Per) x Site (farm); $F$=3.24, $P$=0.024).

**BACI Study: Multivariate analysis of benthic macrofauna assemblages**

The benthic assemblages in the soft sediments under the farm and at the reference locations were compared before and after shell removal. The multivariate analysis revealed that the benthic assemblages were different among nearly all locations (including farm and reference locations) for all time periods (Figure 17). This may be attributed to natural spatial variation in benthic assemblages. An analysis of similarity (ANOSIM) test demonstrated that many locations (both farm and references) had different benthic assemblages before and after the oyster shell was removed from the farm, with some exceptions (Table 2). It was expected that if the farm was having an impact, then the farm assemblages would start to resemble those found at reference locations. Although in one sampling interval after removal, May 08, assemblages were similar among all locations (Global R=-0.006, $P$=0.511), this pattern was not maintained. By November 2008, the benthic assemblages were again dissimilar among all locations.

**Table 2.** Results of the ANOSIM tests (analysis of similarity) comparing the benthic assemblages among all locations for each sampling time in the BACI study. The Global test compares all locations, and the pair-wise tests compare each location to another. *P* value under 0.05 indicates that the compared assemblages are different from one another. For each test 999 permutations were used (random samples selected).

| | **Before** | | | | **After 1** | | | | **After 2** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Time 1** | | **Time 2** | | **Time 3** | | **Time 4** | | **Time 5** | | **Time 6** | |
| | **May 06** | | **Oct. 06** | | **May 07** | | **Sept. 07** | | **May 08** | | **Nov. 08** | |
| | **R** | ***P*** | **R** | ***P*** | **R** | ***P*** | **R** | ***P*** | **R** | ***P*** | **R** | ***P*** |
| ***Global test*** | 0.22 | 0.002 | 0.22 | 0.001 | 0.12 | 0.006 | 0.15 | 0.004 | -0.01 | 0.511 | 0.37 | 0.001 |
| ***Pairwise tests*** | | | | | | | | | | | | |
| **Farm vs Ref. 1** | 0.23 | 0.016 | 0.13 | 0.054 | 0.06 | 0.189 | 0.01 | 0.421 | 0.09 | 0.107 | 0.38 | 0.002 |
| **Farm vs Ref. 2** | 0.02 | 0.356 | 0.14 | 0.048 | 0.08 | 0.147 | 0.30 | 0.001 | 0.02 | 0.357 | 0.66 | 0.001 |
| **Farm vs Ref. 3** | 0.27 | 0.006 | 0.41 | 0.001 | 0.30 | 0.003 | 0.27 | 0.005 | 0.15 | 0.042 | 0.61 | 0.001 |
| **Ref. 1 vs Ref. 2** | 0.24 | 0.003 | 0.01 | 0.381 | 0.10 | 0.088 | 0.06 | 0.172 | -0.13 | 0.979 | 0.20 | 0.002 |
| **Ref. 1 vs Ref. 3** | 0.47 | 0.001 | 0.29 | 0.001 | 0.01 | 0.438 | 0.08 | 0.174 | -0.13 | 0.980 | 0.18 | 0.017 |
| **Ref. 2 vs Ref. 3** | 0.09 | 0.001 | 0.33 | 0.001 | 0.18 | 0.018 | 0.27 | 0.001 | 0.67 | 0.669 | 0.30 | 0.005 |

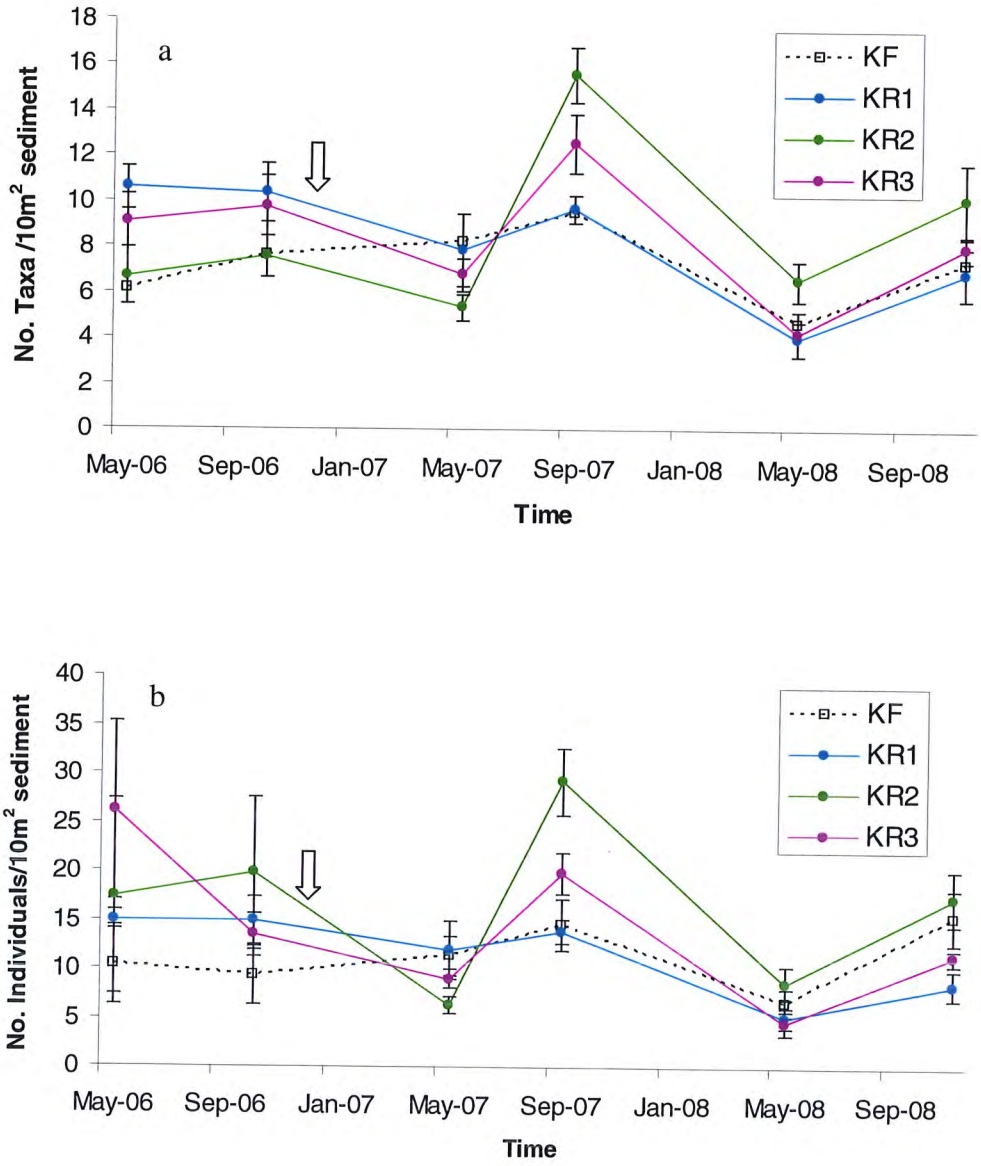


Figure 14. The number of (a) benthic macrofauna taxa and (b) individuals in the soft sediments at Kuri Bay before and after the removal of a pearl farm (arrow indicates date of removal) at both farm (F) and references locations (R).

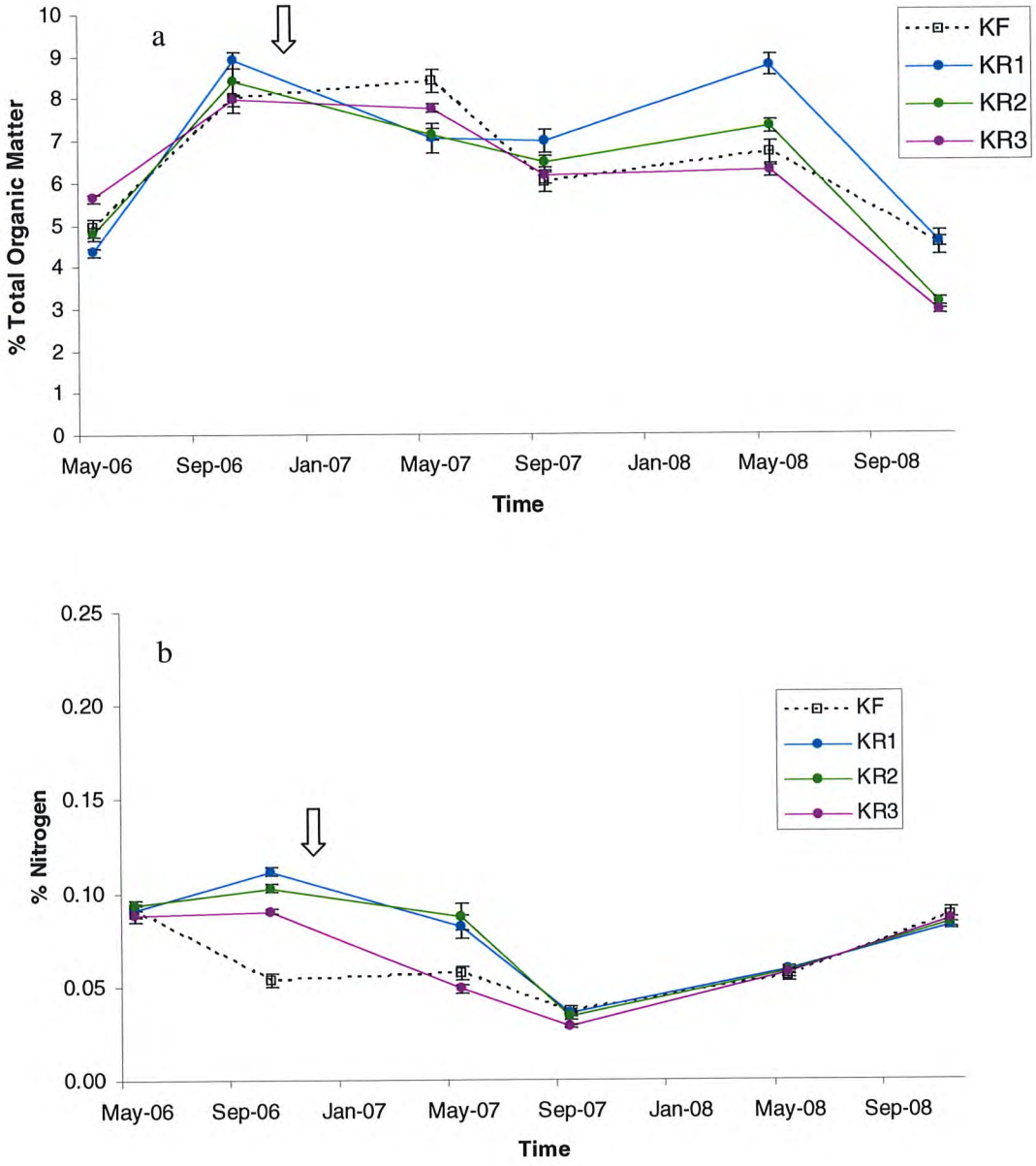


Figure 15. The percentage of (a) total organic matter and (b) nitrogen in the soft sediments at Kuri Bay before and after the removal of a pearl farm (arrow indicates date of removal) at both farm (F) and references locations (R).

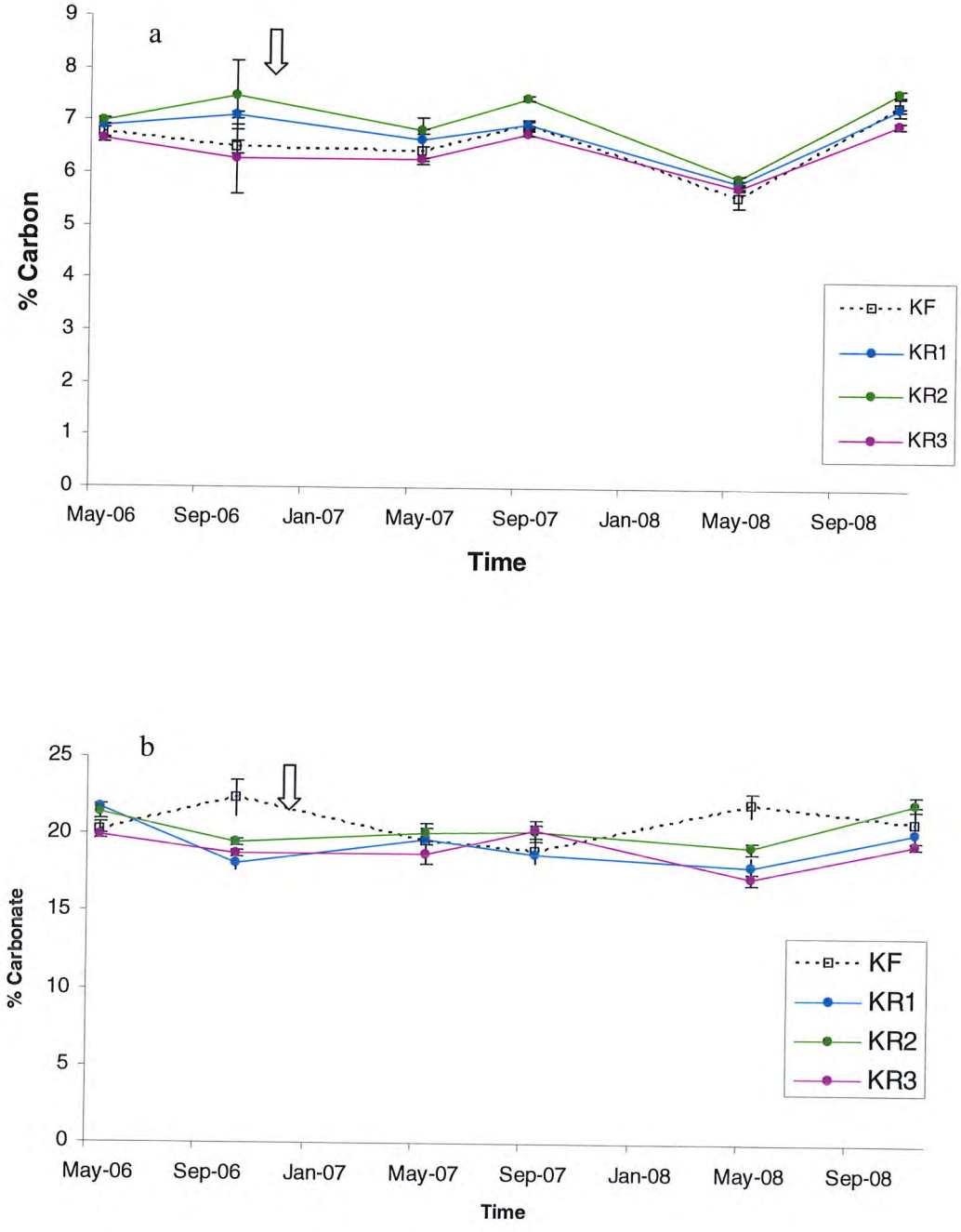


Figure 16. The percentage of (a) carbon and (b) carbonates in the soft sediments at Kuri Bay before and after the removal of a pearl farm (arrow indicates date of removal) at both farm (F) and references locations (R).

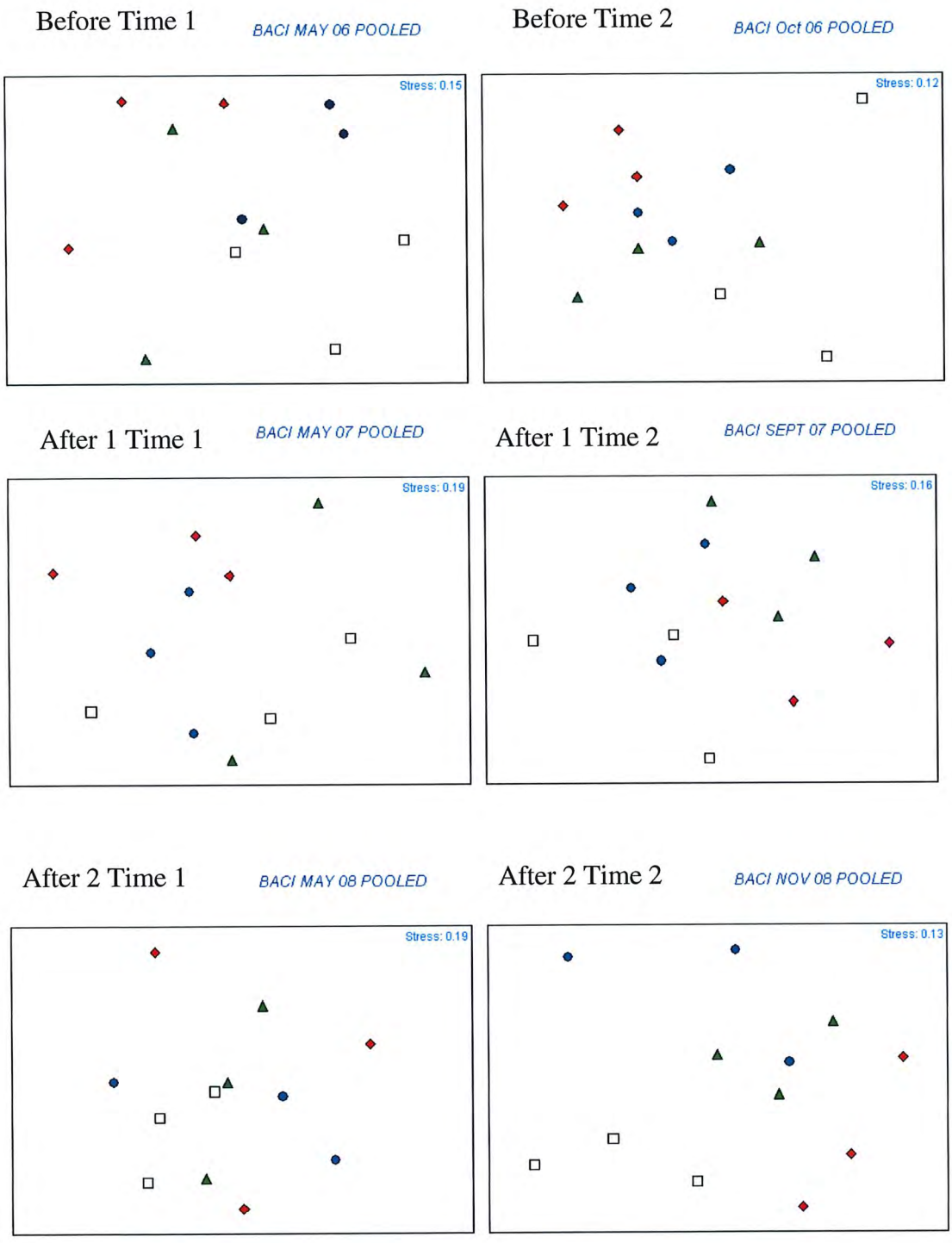


Figure 17. MDS plots of the fauna in farm and references locations over the different sampling periods (Before, After 1 and After 2) and different times within these periods (Time 1 and Time 2). Farms = , Reference , Reference 2 , Reference 3 .

## DISCUSSION

### Main study: Benthic macrofauna (comparison of existing farms with reference locations)

Over two years of benthic sampling has demonstrated that there was no evidence of any consistent change in the total number of benthic macrofauna taxa or individuals within soft sediments that may be directly attributed to pearl oyster longlines compared to reference locations. This outcome is particularly robust because of the rigorous sampling design that was employed to detect changes in benthic macrofauna over three spatial scales (sites, locations, bays) using numerous reference locations (n=4) in each bay and ten random sampling events in time. Too often an environmental impact of shellfish aquaculture (notably mussels) is demonstrated by studies that have only one control (or reference site) or one sampling time (e.g. Kaspar et al 1985, Tenore et al 1985, Grenz et al 1990, Stenton-Dozey et al 1999). Limited spatial or temporal sampling designs are inadequate to demonstrate any potential impact reliably (Underwood 1992) and for these reasons our study sought to undertake the most rigorous sampling protocol to date for assessing potential impacts due to pearl aquaculture.

There was considerable natural variability of the benthic macrofauna among all locations, but especially among the reference locations. The fluctuations in benthic macrofauna found under the longlines at farms were within the bounds of what occurred naturally among reference locations. The reference locations were as different from one another as they were from the farm locations. This indicates that the number of benthic macrofauna taxa, and their relative abundances within sediments underlying the farms fell within the range of natural benthic macrofauna variation found at these spatial scales. In fact, there was greater variability at the site level (50-150m distance) when compared to the variability at the location level (1-5km distance). This can be typical of natural variability in benthic assemblages and corroborates other studies that show small scale variability in fauna can be greater than large-scale variability (Chapman et al 1995, Anderson et al 2005, Norén and Lindegarth 2005). This suggests that for the benthic species of this region, small-scale processes (such as competition, settlement and behaviour) may be more influential than any changes created by pearl farming activities.

We observed different assemblages of benthic macrofauna among bays (separated by 100's of km), as would be expected of different geographical regions. Larger scale processes such as biogeography, climate, and history may drive this variability and contribute to these differences. However, there were no consistent differences in the benthic faunal assemblage under the longlines (at farms) when compared to the reference locations for all times. Therefore, in relation to small and large-scale processes, the farms had no detectable influence on the composition and abundance of benthic fauna in the soft sediments.

The seasonal variability of the fauna is difficult to generalise and tends to be different at each bay. The number of macrofauna taxa appears to peak late in the dry season June to October in Vansittart Bay and Port George but for Cygnet Bay no such pattern appears. Other researchers have found seasonal peaks in tropical fauna. For example, in Darwin Harbour, there was lower diversity of polychaetes in seaward mangrove zones and higher diversity in landward zones during the wet season (Metcalfe and Glasby 2008). Single species analysis may uncover seasonal patterns in distribution and abundance of macrofauna but these will not be discussed for this report. However, even at the single species level, the patterns were highly variable and site specific. For example, hundreds of ovigerous taniads were found in one reference location (from Vansittart Bay) during January 2007 and again in May 2008, but not found at the other reference locations, nor other bays or times. Yet these animals can be generally found in other tropical regions of Australia and the world (Bamber 2005(Goncalves da Costa & Cunha Nalesso, 2006).

**Main study: Sediment physico-chemistry (comparison of existing farms with reference locations)**

When comparing among all bays (3 farms with 12 reference locations), the sediments under the pearl longlines did not exhibit symptoms of nutrient enrichment or eutrophication as found in other aquaculture industries (e.g. fish farms Yokoyama 2002). Overall, the fluctuations of the sediment physico-chemistry under the longlines at the farms were within the bounds of what occurred naturally at the reference locations. Comparisons across all bays revealed no differences between what was observed at the farms compared to the reference locations. In fact, surprisingly very few of the samples taken from under the farms contained shell grit originating from the culture of pearl oyster. This is regardless of the fact that some of the farm sediment samples were taken soon after 'cleaning' of the longlines (removing epiphytic growth from oyster shells, cages and ropes).

When comparing the farm and reference locations within each bay (so comparing one farm with four reference locations) the pattern was somewhat different. In Port George, the farm was found to experience higher levels of nitrogen compared to the reference locations within this bay. Higher nitrogen levels are associated with aquaculture impacts (Christensen et al 2003, Grant et al 1995, Cranford et al 2007) and can potentially be attributed to the deposition of faeces from the cultured pearl oyster on the longlines. However this pattern of elevated nitrogen was not observed at the other farms in Vansittart and Cygnet Bays. The nitrogen levels in Port George underlying the farms also were within the range observed at the reference locations in Cygnet and Vansittart Bays (0.05-0.16 %N). Furthermore, no other signs of nutrient enrichment were detected at the farm in Port George.

The nitrogen levels at Port George (farm average=0.095 %N, range at reference locations=0.07-0.08 %N) were very low compared to levels seen in other marine systems. For example, in a marine embayment of Nova Scotia Canada, the %N levels in sediments underlying mussel farms were ten times the levels recorded in our study (mussel farms=$0.9^{\pm}0.2$ and reference sites=$0.8^{\pm}0.2$, Grant et al 1995). Another study in the same region of Canada found even greater levels of sediment nitrogen ($1.06^{\pm}0.8$ at mussel farms and $0.89^{\pm}0.7$ %N at reference sites, Hargrave et al 1994). In south west Ireland, the %N levels in sediments under mussel farms ranged from $0.58^{\pm}0.08$ to $0.27^{\pm}0.01$ (Chamberlain et al 2001).

It would be expected that if the oysters on the longlines at Port George were raising the nitrogen levels within the underlying sediments then there would be also associated elevations in the sediments of total organic matter and carbon from the deposition of faeces (Christensen et al 2003, Grant et al 1995, Cranford et al 2007). It would also be expected that the benthic macrofauna in these sediments would experience a commensurate change (Pearson and Rosenberg 1978, Grant et al 1995). These changes were not observed at Port George and so it is prudent to note that natural regional variability may be the source of this elevated nitrogen. The lack of sampling before the longlines were established means that it can't be ruled out that the area of the farm at Port George may naturally experience higher nitrogen levels compared to the nearby reference locations even prior to the commencement of oyster farming.

At Vansittart Bay, during one sampling time (Sept. 2007), there were also elevated levels of nitrogen in the sediments underlying the farm compared to the reference locations. However this pattern was not observed at any other time in this bay. Furthermore, during September 2007 in Vansittart Bay there were few other signs of nutrient enrichment. The carbon levels in the sediments in Vansittart Bay were generally lower at the farm compared to the reference locations, which is the opposite of what would be expected from nutrient enrichment. Although the redox potential was in general lower at the farm compared to the reference locations (which can be associated with anoxic sediments and nutrient enrichment), this was not statistically significant, nor was it observed at the other bays. Once again, the lack of sampling before the longlines were established means that this may not associated with farming practices but could be potentially attributed to natural variability.

For the main study, it is also important to note that we are not able to detect any 'pulse' disturbances that may have occurred shortly after (or during) the installation of the longlines; we can only detect long-term 'press' effects in terms of the design of the main study. A 'pulse' disturbance or change in the environment is one in which the disturbance is short term and then removed e.g. accidental oil or chemical spill, or the disturbance created by building of pipeline (Underwood 1992, Glasby and Underwood 1996). The environmental response is usually characterised by large and rapid changes in abundances or other variables (in this case of benthic fauna or sediment physico-chemistry) which then may cause local species extinction or long term environmental changes but there is some chance of recovery because the impact is removed. A 'press' disturbance describes an impact on the environment that is sustained and constant, such as a continuous discharge of sewage or waste (Underwood 1992, Glasby and Underwood 1996). It is called a 'press' disturbance because the impact creates sustained effect on the environment such as decreasing the abundance of benthic fauna (pressing down their numbers). Thus we found little evidence of 'press' related impacts caused by the pearl farms in the main study.

Despite the lack of 'before' monitoring in the main study, we were however, afforded the opportunity to assess the potential effects of pearl aquaculture by monitoring before and after the *removal* of a pearl farm. The design of our Beyond BACI study (Underwood 1992) assumed that the removal of the oyster shell from the pearl farm longlines would cause sustained changes in the benthic fauna and conditions. This farm has been established for over 50 years so we would expect its removal to cause significant long-term changes *if* and

*only if* the farm was creating some change or impact during its operation. The design of the Beyond BACI study allowed us to test for a 'pulse' disturbance; one that may occur soon after pearl oyster removal from the longlines.

**BACI study: Benthic macrofauna (effect of farm closure)**

The removal of the pearl oysters from the longlines did not have an effect on the underlying sediments and benthic fauna, when compared to the natural variability of the sediments at the reference locations. The changes observed between the benthic conditions six months before and then up to two years after the removal of the oyster shells were similar to the natural variability observed at the reference locations in this time. Although the assemblages of benthic macrofauna in this study changed significantly with time, there were no consistent changes in the benthic fauna assemblages that could be attributed to the removal of shell from the longlines.

**BACI study: Sediment nutrient levels (effect of farm closure)**

The fluctuations of the sediment nutrient levels (total organic matter, carbon, nitrogen and carbonates) under the longlines at the farm were within the bounds of what occurred naturally at the reference locations, both before and after oyster shell removal. There were no differences between what was observed in the sediments at the farm compared to the reference locations, or any significant differences before and after shell removal at the farm. Similar to the main study, the sediments under the pearl longlines did not exhibit evidence of nutrient enrichment or eutrophication.

However in October 2006 (before shell removal), we found that the pearl farm sediments had lower nitrogen levels than the reference locations. This lower level of nitrogen was not sustained at other sampling times. Generally, recorded effects of shellfish and finfish aquaculture in other regions of the world are to enhance sediment nitrogen levels (Christensen et al 2003, Grant et al 1995, Cranford et al 2007). Therefore, detecting lower levels of nitrogen in the sediments under the pearl longlines was unexpected. This pattern was not observed six months later in the next sampling time and may be attributed to the natural sediment variability observed in this region.

**No dominance of indicator species of organic enrichment**

Benthic fauna has been used as an indicator of organic enrichment (from anthropogenic sources) particularly the Capitellid polychaetes, some Spionid polychaetes and gastropod molluscs. In fact 17 polychaete and 7 mollusc groups have been historically used as indicators of organic enrichment (Pearson and Rosenberg 1978), however none of these studies were from the Indo-Pacific region and so direct comparisons with our study are limited. Yet we did find that the benthic assemblage in the Kimberley, both at the pearl farms and reference locations, was very diverse and not dominated by one species or one group of taxa. In other studies of sediments affected by nutrient enrichment (or other environmental impacts), one or a few species or groups of taxa tend to dominate the sediments (Pearson and Rosenberg 1978, Stenton-Dozey et al 1999, Callier et al 2007). In particular for shellfish aquaculture, the use of ropes, racks buoys and lines is expected to have an immediate effect on local hydrography and provide a new substratum upon which other epibiota can attach and grow (Goncalves da Costa and Cunha Nalesso 2006). This can then potentially lead to a new or changed benthic fauna occurring in the sediments underneath the longlines.

Changes to polychaete assemblages are recognised as one of the best indicators of environmental impacts from aquaculture (Pearson and Rosenberg 1978, Hutchings 1998). Therefore the presence or absence of specific polychaetes in marine sediments can provide an indication of the condition or health of the benthic environment (Pocklington and Wells 1992). For example, some polychaetes families, such as those belonging to the Capitellidae (i.e. *Capitella capitata*), Cirratilidae, and Spionidae families will dominate benthic communities in sediments experiencing excessive organic enrichment (Hutchings 1998, Giangrande et al 2005, Surugiu 2005, Cardoso et al 2007). Although some of these groups were collected in the sediments (Main study: Capitellids n=311, Cirratilids n=495, and Spionids n=186) they were collected from the sediments of both farm and reference locations and potentially reflect a naturally occurring population of fauna. There were no differences in their numbers between farm and reference locations (Capitellids; mean number per grab at farms=$1.40^{\pm}0.05$, references=$1.65^{\pm}0.09$, Cirratilids mean no. per farms=$1.08^{\pm}0.06$, references=$2.03^{\pm}0.06$, Spionids mean no. per farms=$1.05^{\pm}0.05$, references=$1.25^{\pm}0.02$).

In contrast, some polychaete families can experience a decline in numbers during anthropogenic impacts. For example, the family Syllidae have shown to be a very useful indicator taxon in hard substrata as they are highly sensitive to pollution and disturbances, decreasing in numbers of species and individuals or completely disappearing in adverse

conditions (Giangrande et al 2005). However in our study there were similar numbers of Syllids at the farms compared to the reference locations (mean no. per grab at farms=8.95±2.67, references=8.33±1.24). Although we sampled in soft sediments and not hard substrata, the abundance of Syllids in the sediments under the longlines could suggest an absence of disturbance occurring under the pearl farms.

The mollusc species collected in our study reflected the normal fauna of tropical north Australia and only one genus *Macoma* sp. was collected from the Kimberley that has been identified by Pearson and Rosenberg (1978) as an indicator of organic enrichment. However this mollusc was found in similar abundances at the pearl farms and reference locations (mean no. per grab at farms=0.16±0.004, references=0.18±0.001).

**Influence of hydrodynamic regime**

In New Zealand, the influence of longline mussel aquaculture on benthic conditions has been demonstrated to be correlated with the hydrodynamic activity (e.g. water currents, tidal regime, and wave action) of the area in which the farm is located (Hartstein and Rowden 2004). In low hydrodynamic energy sites (mean current velocity 3.16-4.09 cm $s^{-1}$), the benthic macrofauna assemblages under the farm were different to those at reference sites located away from the farm (up to 200m). However, in high energy sites (mean current velocity 9.7-10.2 cm $s^{-1}$) there was no difference in the benthic assemblages between reference and farm sites. In low energy sites, the mussel longlines were found to drop mussel shell debris to the underlying sediments which were also higher in %TOM than the reference sites. This increased shell grit and %TOM in the sediment was correlated with a change in benthic macrofauna (Hartstein and Rowden 2004). In high energy sites, the hydrodynamic activity (tides, water currents) would sweep away this accumulation of debris and no differences in the benthic conditions between reference and farm sites were found.

Similar results have been found by other researchers when comparing low to high hydrodynamic energy sites (Chamberlain et al 2001). They found at the low energy site (mean current velocity 2.28-2.85 cm $s^{-1}$) the farm was subjected to organic enrichment, reduced macrofauna diversity and high sediment carbon compared to the three reference stations. This impact however, was restricted to a radius of only 40m around the farm. Conversely, at the high energy site (mean current velocity 3.11- 3.14 cm $s^{-1}$), the farm was not different from the nearby reference stations and contained a diverse macrobenthic

community. The local water currents and associated hydrodynamic dispersion of biodeposits was considered responsible for 'cleaning up' the high energy farm site.

An energetic hydrodynamic regime can compensate for potential impacts of aquaculture ventures. For example, in a Brazilian estuary that had shallow water depths (average 3m) and sediments with a high percentage of silt-clay, the mussel farms were not found to have any deleterious effects because of the high hydrodynamic flow of the Benevente River estuary (Goncalves da Costa and Cunha Nalesso, 2006). The currents dispersed the organic matter, mussel shells and fallen fouling organisms from the farms as no traces of these were found under the farm lines although they were in only 3 m water depth.

In our study, the pearl farms are located in much deeper water (range 10-16m) and experience diurnal macro-tidal regimes so it is not surprising that we too, did not find any accumulation of organic deposits or shell debris. For this reason we suspect that the pearl farms of the Kimberley are unlikely to create environmental issues as seen in other regions of the world that have lower energy hydrodynamic regimes (e.g. Tenore 1985, Chamberlain et al 2001). However it would be useful for the industry to have some measure of the hydrographical conditions in the area. A review of the literature suggests that it would be prudent for future proposed pearl farms to be located in areas that have greater than 5 cm $s^{-1}$ average water currents to avoid biodeposit accumulation.

**Comparison of our studies with other shellfish studies**

In general, there is a lack of consensus regarding the environmental effects of shellfish aquaculture and this is not surprising given the different ecosystems and conditions that shellfish farms are located in. Furthermore the husbandry practices of each farm may be very different, and these can, in turn, influence potential effects of the farm on the natural environment. A review of the scientific literature suggests that the main mechanisms influencing the impact of shellfish aquaculture include; the farming method, the density of the cultivated shellfish (or stocking rate), the water depth of the farm area and, as mentioned above, the hydrographical conditions in the area (Danovaro et al 2004). All these factors can limit the comparisons made between other aquaculture studies and therefore between farms.

In summary, there are numerous studies that suggest shellfish aquaculture can produce organic enrichment and alteration of benthic macrofauna (Mattsson & Linden 1983, Kaspar

et al 1985, Tenore et al 1985, Stenton- Dozey et al 1999, Mirto et al 2000, Callier et al 2007) however half of these studies had limited spatial and temporal replication. In contrast, other studies suggest shellfish aquaculture to have little or no impacts (Hatcher et al 1994, Danovaro et al 2004, Goncalves Da Costa and Cunha Nalesso 2006, Lasiak et al 2006, Mallet et al 2006) while others suggest that the conditions of the aquaculture and its environment can determine whether an impact occurs or not. For example, In Nova Scotia, one study found some biodeposition occurring under mussel lines compared to reference sites, but the sediments were not anoxic, and a diverse and active benthic community persisted regardless.

In conclusion, the influences of pearl oyster aquaculture (in the Kimberley) cannot be assumed nor extrapolated from the numerous studies to date assessing mussel aquaculture impacts. The environmental influences of pearl oyster farming on benthic conditions appear to be indistinguishable from natural background variability (as proposed by Enzer MEC 1998). We suggest that the industry is sustainable because the deposition of oyster biodeposits must be within the limitations of the assimilation and dispersal properties of the surrounding environment (Lasiak et al 2006).

**Conclusion**

The pearl farming in the Kimberley region can be considered ecologically sustainable in terms of its lack of observed impacts on benthic macrofauna and sediment physico-chemistry. We suspect that the hydrodynamic activity of the region is so energetic that there is low concern regarding the accumulation of organic debris and nutrients in the sediments underlying the farms. This study has been exhaustive in the design of the sampling regime; and employed three spatial scales and random temporal sampling. We have used a multi-control sampling strategy, to give an estimate of the natural variability of the variables measured. We have also used a Beyond BACI (Underwood 1991, 1992) approach to investigate the effects of removing a pearl farm that had been established for over 50 years. These multiple lines of evidence all conclude that variability in benthic conditions beneath farms in the region are within the bounds of natural variability at other locations.

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**APPENDIX ONE:** Fauna collected in both studies from the sediments of the Kimberley coastal region.

**Table A1.1A.** The diversity of phylum **Annelida**: class **Polychaeta**, collected in the main study.

| Order | Family | Oct. 06 | Jan. 07 | May 07 | Sept 07 | Nov. 07 | Feb. 08 | Apr. 08 | May 08 | Aug. 08 | Nov. 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amphinomida | Amphinomidae | 83 | 68 | 40 | 62 | 104 | 61 | 74 | 77 | 83 | 105 | **757** |
| Capitellida | Capitellidae | 28 | 44 | 25 | 49 | 24 | 25 | 42 | 14 | 17 | 43 | **311** |
| Capitellida | Maldanidae | 138 | 96 | 31 | 78 | 18 | 17 | 39 | 4 | 4 | 26 | **451** |
| Eunicida | Eunicidae | 52 | 23 | 33 | 48 | 38 | 20 | 28 | 24 | 36 | 48 | **350** |
| Eunicida | Lumbrineridae | 34 | 12 | 13 | 18 | 19 | 6 | 9 | 6 | 5 | 10 | **132** |
| Eunicida | Oenonidae | | 2 | 1 | | | | | | 2 | 1 | **6** |
| Eunicida | Onuphidae | 47 | 16 | 11 | 3 | 4 | 4 | 5 | 3 | 6 | 4 | **103** |
| Flabelligerida | Flabelligeridae | 48 | 25 | 9 | 20 | 57 | 23 | 14 | 10 | 1 | 8 | **215** |
| Opheliida | Opheliidae | | 6 | 1 | 4 | 2 | | 1 | 2 | 5 | 5 | **26** |
| Orbiniida | Orbiniidae | 1 | 5 | | | | | | 1 | 4 | | **11** |
| Orbiniida | Paraonidae | 8 | 5 | 13 | 14 | 20 | 5 | | 1 | 5 | 9 | **80** |
| Phyllodocida | Acoetidae | | | 2 | 1 | | | | | | | **3** |
| Phyllodocida | Aphroditidae | 1 | | | | | | | | 1 | | **2** |
| Phyllodocida | Chrysopetalidae | 1 | 7 | 2 | 3 | 4 | | 2 | 7 | 4 | 3 | **33** |
| Phyllodocida | Glyceridae | 9 | 14 | 10 | 9 | 7 | 3 | 5 | 8 | 15 | 7 | **87** |
| Phyllodocida | Goniadidae | | 3 | 2 | | 3 | 2 | 1 | 3 | 3 | 3 | **20** |
| Phyllodocida | Hesionidae | 1 | | 3 | 2 | 3 | 3 | 4 | 5 | 2 | 2 | **25** |
| Phyllodocida | Nephtyidae | 23 | 19 | 15 | 9 | 10 | 3 | 8 | 10 | 17 | 13 | **127** |
| Phyllodocida | Nereididae | 9 | 27 | 6 | 10 | 8 | 6 | 2 | 10 | 6 | 27 | **111** |
| Phyllodocida | Paralacydoniidae | | 9 | 1 | 1 | 1 | 1 | 1 | | | | **14** |
| Phyllodocida | Phyllodocidae | 3 | 4 | 8 | 10 | 6 | 7 | 6 | 5 | 5 | 8 | **62** |
| Phyllodocida | Pilargidae | 6 | 4 | 16 | 17 | 29 | 15 | 19 | 25 | 13 | 43 | **187** |
| Phyllodocida | Polynoidae | 14 | 10 | 5 | 13 | 9 | 9 | 12 | 11 | 9 | 17 | **109** |
| Phyllodocida | Sigalionidae | 65 | 22 | 20 | 41 | 26 | 12 | 14 | 11 | 25 | 27 | **263** |
| Phyllodocida | Syllidae | 111 | 21 | 5 | 9 | 8 | 91 | 8 | 161 | 89 | 7 | **510** |
| Sabellida | Sabellidae | 16 | 6 | 18 | 39 | 39 | 61 | 41 | 19 | 24 | 34 | **297** |
| Spionida | Chaetopteridae | 7 | 5 | 118 | 23 | 41 | 12 | 31 | 17 | 19 | 18 | **291** |
| Spionida | Cirratulidae | 60 | 130 | 32 | 52 | 37 | 41 | 44 | 19 | 37 | 43 | **495** |
| Spionida | Magelonidae | 1 | | 2 | | 10 | 10 | 4 | 7 | 16 | 16 | **66** |
| Spionida | Poecilochaetidae | 1 | | 7 | 6 | 1 | 1 | | 3 | 1 | 4 | **24** |
| Spionida | Spionidae | 14 | 15 | 27 | 13 | 15 | 8 | 31 | 20 | 22 | 21 | **186** |
| Sternaspida | Sternaspidae | 52 | 19 | 17 | 42 | 16 | 12 | 22 | 14 | 28 | 26 | **248** |
| Terebellida | Ampharetidae | 11 | 94 | 12 | 27 | 17 | 4 | 20 | 6 | 8 | 7 | **206** |
| Terebellida | Pectinariidae | 8 | 1 | 1 | 11 | 2 | 2 | | 1 | 11 | 10 | **47** |
| Terebellida | Sabellariidae | 2 | | | 1 | | | | 1 | 1 | 1 | **6** |
| Terebellida | Terebellidae | 60 | 42 | 41 | 139 | 102 | 85 | 109 | 62 | 85 | 140 | **865** |
| Terebellida | Trichobranchidae | 310 | 62 | 43 | 158 | 193 | 55 | 9 | 1 | 22 | 83 | **936** |

**Table A1.1B.** The diversity of phylum **Arthropoda**: subphylum **Crustacea**, class Malacostraca (M) or Ostracoda (O) collected in the main study (Orders: Amp= Amphipoda, Cum= Cumacea, Dec= Decapoda, Iso= Isopoda, Tan=Tanaidacea), (Sub-Infraorders: Brach=Brachyura, Car= Caridea, Dend=Dendobranchiata).

| Class | Order | Sub Infra Order | Family Superfamily | Genus, Species or common name | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | Amp | | Corophiidae | *Cheiriphotis megacheles* | | 7 | | | | | | | | | **7** |
| M | Amp | | Corophiidae | Corophiid sp. 1 | 22 | 12 | 26 | 21 | 18 | 40 | 78 | 25 | 21 | 25 | **288** |
| M | Amp | | Corophiidae | Corophiid sp. 2 | | | 1 | 7 | 2 | | 2 | 2 | 10 | 1 | **25** |
| M | Amp | | Corophiidae | Corophiid sp. 3 | | | | | | 2 | 3 | | 2 | | **7** |
| M | Amp | | Phoxocephalidae | Phoxo. sp. 1 | 18 | 21 | 7 | 8 | 12 | 4 | 11 | 6 | 4 | 16 | **107** |
| M | Amp | | Phoxocephalidae | Phoxo. sp.2 | | | | | 1 | 2 | | 1 | 1 | 2 | **7** |
| M | Amp | | Aoridae | Aoridae sp. 1 | 69 | 10 | 5 | 15 | 5 | 7 | 3 | 8 | 5 | 6 | **133** |
| M | Amp | | Aoridae | Aoridae sp. 2 | | | | | | 3 | 6 | 6 | 4 | 7 | **26** |
| M | Amp | | Caprellidae | Phtisicinae | 5 | 29 | 7 | 14 | 6 | | 3 | 2 | 4 | 14 | **84** |
| M | Amp | | Caprellidae | Caprellidae sp. 2 | | | | | 2 | | 1 | | 1 | 3 | **7** |
| M | Amp | | Lysianassidae | *Acidostoma sp.* | 20 | 9 | 9 | 10 | 3 | 2 | 5 | 8 | 14 | 20 | **100** |
| M | Amp | | Ampeliscidae | | 4 | 17 | 17 | 29 | 10 | 10 | 19 | 13 | 25 | 22 | **166** |
| M | Amp | | Dexaminidae | Dexaminid sp. 1 | 1 | 6 | | | 5 | 1 | | | | | **13** |
| M | Amp | | Dexaminidae | *Polychuria* sp. | | | 7 | | 9 | | | | | 1 | **17** |
| M | Amp | | Corophoidea | | 3 | 28 | 3 | | | | | | | | **34** |
| M | Amp | | Leucathoidae | Leucathoid sp. 1 | 8 | | 2 | 4 | | | 5 | | 3 | 1 | **23** |
| M | Amp | | Leucathoidae | Leucothoid sp. 2 | | 4 | | 2 | | 1 | | | 1 | | **8** |
| M | Amp | | Leucathoidae | Leucothoid sp. 3 | | | | | | | 2 | 1 | 3 | 1 | **7** |
| M | Amp | | Leucathoidae | Leucathoid sp. 4 | | | | | | | | 1 | | | **1** |
| M | Amp | | Stenothoidae | Stenothoid sp. 1 | 1 | | 1 | 1 | | | 8 | 5 | 1 | 3 | **20** |
| M | Amp | | Stenothoidae | Stenothoid sp. 2 | | | | | | | 13 | | 1 | 3 | **17** |
| M | Amp | | Podoceridae | Podocerid sp. 1 | 1 | 10 | 4 | 5 | 13 | 11 | 10 | 56 | 6 | 9 | **125** |
| M | Amp | | Podoceridae | Podocerid sp.2 | | | | | | | 1 | 22 | | | **23** |
| M | Amp | | Melitidae | *Malacoota chandaniae* | 4 | 16 | 4 | 4 | 4 | 2 | | 2 | | 1 | **37** |
| M | Amp | | Melitidae | *Mallacoota subinsignis* | | | 3 | | | | | | 1 | | **4** |
| M | Amp | | Liljeborgiidae | Liljeborgiid sp. 1 | | | | 1 | | 1 | 2 | 2 | 1 | 3 | **10** |
| M | Amp | | Liljeborgiidae | Liljeborgiid sp. 2 | | | | 1 | 1 | | | 3 | | 1 | **6** |
| M | Cum | | Bodotriidae or Diastylidae | | 29 | 23 | 12 | 41 | 11 | 6 | 7 | 4 | 25 | 30 | **188** |
| M | Dec | Brach | | Crab megalopa | | | 2 | 4 | 3 | | | 3 | 1 | | **13** |
| M | Dec | Brach | | Crab zoae | | | 3 | 3 | 1 | 1 | 5 | 1 | | | **14** |
| M | Dec | Brach | Dorripidae | *Paradorippe australiensis* | | | 2 | | | | | | | | **2** |
| M | Dec | Brach | Ebaliinae | Ebaliinae sp. 1 | | | 1 | | | | | 1 | | | **2** |
| M | Dec | Brach | Ebaliinae | Ebaliinae sp. 2 | | | | 4 | | 2 | 1 | | | 1 | **8** |
| M | Dec | Brach | Goneplacidae | Goneplacid sp. 1 | | 2 | 2 | 1 | 4 | 2 | 5 | 3 | 4 | 2 | **25** |
| M | Dec | Brach | Goneplacidae | Goneplacid sp. 2 | 5 | | 2 | | 1 | | 2 | 2 | 2 | 3 | **17** |
| M | Dec | Brach | Hexapodidae | | 7 | 6 | 1 | 2 | 1 | | | 2 | | 1 | **20** |
| M | Dec | Brach | Leusosiidae | | | 1 | 1 | 2 | | | 2 | 1 | 1 | | **8** |
| M | Dec | Brach | Majidae | | | | 1 | | | | 1 | | | | **2** |
| M | Dec | Brach | Ocypodidae | | 8 | 1 | | 2 | | | | | | | **11** |

**Table A1.1B** continued. The diversity of **Arthropoda**: subphylum **Crustacea**.

| Class | Order | Sub Infra Order | Family Superfamily | Genus, Species or common name | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 1 | 4 | 2 | 3 | | 1 | 7 | 11 | 4 | 5 | 6 | **43** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 2 | | 2 | 3 | 5 | 2 | 3 | 9 | 3 | 2 | 2 | **31** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 3 | 1 | | | | 5 | 1 | 2 | | | 14 | **23** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 4 | | | | | 1 | 2 | 1 | 1 | 3 | 4 | **12** |
| M | Dec | Brach | Pilumnidae | Rhizopinae | 30 | 15 | 18 | 18 | 16 | 12 | 17 | 15 | 8 | 18 | **167** |
| M | Dec | Brach | Pilumnidae | *Ceratoplax* sp. | 2 | | 4 | 2 | 3 | 2 | 2 | 3 | | 2 | **20** |
| M | Dec | Brach | Pilumnidae | *Cryptolutea arafurensis* | 2 | 3 | 2 | | | 1 | 2 | | 1 | 1 | **12** |
| M | Dec | Brach | Pinnotheridae | | | 2 | 1 | | 2 | | 1 | | 1 | | **7** |
| M | Dec | Brach | Porcellanidae | | | | 1 | | 1 | | | | | | **2** |
| M | Dec | Brach | Portunidae | *Podophthalmus* sp. | | 3 | 2 | | 2 | | | | | | **7** |
| M | Dec | Brach | Portunidae | *Thalamita* sp. | 3 | | 6 | 3 | 5 | 4 | 3 | 3 | 2 | 5 | **34** |
| M | Dec | Brach | Xanthidae | | | | | 1 | | | | | | | **1** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 1 | 5 | 3 | 6 | 2 | 2 | 3 | 12 | 11 | 6 | 1 | **51** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 2 | 1 | | | | 3 | | 3 | 3 | | 1 | **11** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 3 | 1 | | | 1 | 2 | | | 1 | | 3 | **8** |
| M | Dec | Carid | Axiidae | *Paraxopsis dianae* | | 5 | 4 | 7 | 2 | 1 | 3 | 1 | 2 | 18 | **43** |
| M | Dec | Carid | Crangonidae | *Philocheras* sp. | 1 | 1 | | 2 | | 1 | 3 | 2 | 3 | 6 | **19** |
| M | Dec | Carid | Hypolytidae | *Alopes* sp. 1 | | | 2 | | 1 | 1 | | | | 1 | **5** |
| M | Dec | Carid | Hypolytidae | *Alopes* sp. 2 | 1 | | 1 | | | | | | 1 | 2 | **5** |
| M | Dec | Carid | Ogyrididae | *Ogyrides* sp. | 29 | 17 | 42 | 34 | 16 | 30 | 39 | 41 | 47 | 27 | **322** |
| M | Dec | Carid | Pandalidae | *Chlorotocella* sp. | | 2 | | | | | | | | | **2** |
| M | Dec | Carid | Pasiphaeidae | *Leptochela sydniensis* | 13 | 7 | 9 | 16 | 15 | 20 | 32 | 30 | 20 | 7 | **169** |
| M | Dec | Carid | Processidae | *Processa longirostris* | 1 | 12 | 4 | | 3 | 6 | 7 | 7 | 2 | 2 | **44** |
| M | Dec | Dend | Luciferidae | | 6 | 1 | 17 | 5 | 5 | 6 | 6 | 3 | 1 | | **50** |
| M | Dec | Dend | Penaeidae | | 6 | 7 | 8 | 5 | 3 | 5 | 4 | 3 | 8 | 3 | **52** |
| M | Dec | Dend | Sergestidae | *Sergia* sp. | | | | 1 | 1 | | | | | | **2** |
| M | Iso | | Anthuridae | *Amakusanthura* sp. | 4 | 9 | 5 | 9 | 8 | 2 | 4 | 10 | 14 | 14 | **79** |
| M | Iso | | Arcturidae | *Neastacilla* sp. | | 4 | | | | | | | 2 | | **6** |
| M | Iso | | Cirolanidae | | 5 | 2 | 2 | 4 | 4 | 7 | 2 | | 3 | 1 | **30** |
| M | Iso | | Gnathiidae | *Gnathia* sp. | 1 | 3 | 1 | 2 | | 1 | | | 4 | | **12** |
| M | Iso | | Serolidae | *Serolina* sp. | 1 | | | 1 | | | | | 1 | 2 | **5** |
| M | Iso | | Spaeromatidae | | | | 1 | | | | 3 | | 3 | | **7** |
| M | Mysidacea | | | | 2 | 8 | | 1 | | | | | | | **11** |
| M | Nebaliacea | | | *Nebalia* sp. | | | | 1 | | 1 | 3 | 9 | 3 | 6 | **23** |
| M | Stomapoda | | | | 3 | 1 | 4 | 6 | | 3 | 5 | 2 | 1 | 3 | **28** |
| M | Tan | Apseudomorpha | | *Saltipedis /Whiteleggia* sp. | 349 | 539 | 55 | 205 | 87 | 73 | 283 | 425 | 233 | 134 | **2383** |
| M | Tan | Tanaidomorpha | | *Leptochelia /Bathytanais* sp. | 5 | 4 | 1 | 18 | 3 | | 2 | 4 | 4 | 2 | **43** |
| O | | | | Ostracoda sp. 1 | 47 | 25 | 12 | 33 | 16 | 11 | 14 | 7 | 14 | 30 | **209** |
| O | | | | Ostracoda sp. 2 | | | 5 | | 2 | 1 | 5 | 5 | 5 | 8 | **31** |

**Table A1.1C.** The diversity of phylum **Mollusca**: class **Bivalva (B) or Gastropoda (G)**, collected in the main study.

| Class | Order | Family | Genus or Species | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B | Arcoida | Arcidae | *Anadara* sp. 1 | 2 | | 1 | 1 | | | 2 | | 1 | | **7** |
| B | Arcoida | Arcidae | *Baritia barbitia* | | | | | 1 | | | | | | **1** |
| B | Arcoida | Limopsidae | *Limopsis* sp. | | | | | | | 1 | | | | **1** |
| B | Myoida | Corbulidae | *Anisocorbula macgillivrayi* | 2 | 7 | 5 | 9 | 4 | 2 | 10 | 1 | 7 | 3 | **50** |
| B | Myoida | Corbulidae | *Notocorbula fortisulcata* | 3 | 2 | 2 | 10 | 7 | 2 | 60 | 80 | 117 | 26 | **309** |
| B | Myoida | Corbulidae | *Serracorbula solidula* | 9 | 5 | 6 | 24 | 15 | 7 | 27 | 13 | 28 | 12 | **146** |
| B | Mytiloida | Mytelidae | *Arcuatula* sp. | 20 | 1 | 1 | 10 | 1 | 9 | 5 | 8 | 14 | 7 | **76** |
| B | Nuculoida | Nuculanidae | *Nuculana* c.f. *darwini* | 8 | 9 | 1 | 11 | 5 | 7 | | 2 | 1 | 8 | **52** |
| B | Nuculoida | Nuculanidae | *Nuculana* sp. 1 | 1 | 5 | 4 | 14 | 2 | | | 4 | 3 | | **33** |
| B | Nuculoida | Nuculanidae | *Nuculana* c.f.*electiliis* | | 2 | | | 1 | | | 2 | | 1 | **6** |
| B | Nuculoida | Nuculanidae | *Nuculana* sp. 3 | | | | 1 | 1 | 2 | | 1 | 1 | 1 | **7** |
| B | Nuculoida | Nuculanidae | *Yolda narthecia* | 1 | 1 | 3 | 7 | 4 | | | 1 | | | **17** |
| B | Nuculoida | Nuculanidae | *Yoldia lata* | | 1 | | 6 | | 6 | 7 | 2 | 2 | | **24** |
| B | Nuculoida | Nuculidae | *Leionucula cumingi* | 8 | 9 | 3 | 27 | 10 | 8 | 7 | 3 | 8 | 2 | **85** |
| B | Nuculoida | Nuculidae | *Leionucula definata* | 5 | 3 | | 6 | | 9 | 9 | 9 | 9 | 11 | **61** |
| B | Nuculoida | Nuculidae | *Leionucula obliqua* | 2 | 2 | | | | 2 | 9 | 5 | 3 | 10 | **33** |
| B | Nuculoida | Nuculidae | *Nucula* sp. 2 | 8 | 2 | 4 | 9 | 2 | | | | | | **25** |
| B | Nuculoida | Nuculidae | *Nuculid* sp. 1 | 4 | 10 | 5 | | 2 | | | | | | **21** |
| B | Pholado-myoida | Laternulidae | *Laternula* c.f. *attenuata* | 2 | | 1 | 2 | | 1 | 2 | | | 2 | **10** |
| B | Pholado-myoida | Myochamidae | *Myadora sp.* | | | 3 | 2 | 1 | 3 | 2 | 3 | | | **14** |
| B | Pterioida | Limidae | *Limaria* c.f. *fragilis* | 1 | | | 1 | | | | | | | **2** |
| B | Solemyoida | Solemyidae | *Theora fragilis* | 13 | 14 | 167 | 45 | 24 | 42 | 205 | 165 | 145 | 26 | **846** |
| B | Veneroida | Galeommatidae | *Curvemysella* c.f. *paula* | 12 | 9 | 3 | | | | 1 | 2 | | | **27** |
| B | Veneroida | Lucinidae | *Anodontia* sp. | 8 | 25 | 50 | 73 | 19 | 62 | 30 | 73 | 99 | 3 | **442** |
| B | Veneroida | Lucinidae | *Cardiolucina eucosmia* | 100 | 83 | 47 | 96 | 58 | 90 | 66 | 29 | 51 | 20 | **640** |
| B | Veneroida | Lucinidae | *Lucine* sp. 1 | 12 | 5 | 8 | 17 | 7 | 6 | 15 | 10 | 11 | 17 | **108** |
| B | Veneroida | Pharidae | *Nucula torresi* | | | | 2 | | 3 | 2 | 2 | 2 | 1 | **12** |
| B | Veneroida | Semelidae | *Sinonovacula constricta* | 4 | | | | 1 | | 2 | | 1 | 2 | **10** |
| B | Veneroida | Tellinidae | *Macoma* sp. 1 | 21 | 11 | 3 | 16 | 5 | 19 | 4 | 3 | 32 | 12 | **126** |
| B | Veneroida | Tellinidae | *Macoma* sp. 2 | 44 | 64 | 21 | 77 | 22 | 23 | 48 | 22 | 23 | 28 | **372** |
| B | Veneroida | Tellinidae | *Solenmyid* sp. 1 | 3 | 5 | 1 | 9 | 3 | 5 | 7 | | | | **33** |
| B | Veneroida | Tellinidae | *Tellina* sp 1 | | | | | | | | | 1 | | **1** |
| B | Veneroida | Veneridae | *Paphia undulata* | 19 | 14 | 12 | 13 | 9 | 12 | 41 | 30 | 14 | 7 | **171** |
| B | Veneroida | Veneridae | *Pitar* sp. | 7 | 5 | 5 | 13 | 1 | 9 | 3 | 8 | 11 | 15 | **77** |
| B | Veneroida | Veneridae | *Placamen callophylum* | | 1 | 6 | | | 1 | 1 | 1 | 1 | 1 | **12** |
| B | Veneroida | Veneridae | *Venerid* sp. 1 | 4 | 2 | 2 | 1 | 2 | 4 | | | | 1 | **16** |
| B | Veneroida | Veneridae | *Venerid* sp. 2 | | | | | 1 | | | | | 5 | **6** |
| B | Veneroida | Veneridae | *Venerid* sp. 3 | | | | | 2 | | | | | | **2** |
| G | | Acteonidae | *Pupa* sp. | | | | | 1 | | | | | | **1** |
| G | | Cerithiidae | *Cerithium* sp. 2 | | | 2 | 2 | 1 | 2 | | | 2 | | **9** |
| G | | Cerithiidae | *Cerithium tirosii* | 2 | 18 | 1 | 3 | 7 | 13 | 3 | 17 | 3 | 6 | **73** |
| G | | Columbellidae | *Zafra* sp. 1 | 4 | 2 | 5 | 16 | 5 | 4 | 5 | 11 | 7 | 2 | **61** |
| G | | Cylichnidae | *Cylichna* sp. 1 | 4 | 2 | 3 | 5 | 1 | 4 | 3 | 10 | 6 | 5 | **43** |
| G | | Cylichnidae | *Cylichna* sp. 2 | | | | | | | | | | 2 | **2** |

**Table A1.1C** continued. The diversity of phylum **Mollusca** collected in the main study .

| Class | Family | Genus or Species | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | Cystiscidae | *Granulina anxia* | | | | | | 1 | | 2 | 1 | | **4** |
| G | Dentaliidae | *Dentalium* sp. | 4 | 2 | 1 | 7 | 2 | 4 | 16 | 2 | 2 | 1 | **41** |
| G | Epitoniidae | *Epitonium* sp. | | | | | | 1 | | | | | **1** |
| G | Gastropteridae | *Siphopteron* sp. | 1 | | 1 | | | | | | | | **2** |
| G | Haminolidae | *Mnestia bizona* | 1 | 2 | | | | | 1 | 1 | 1 | | **6** |
| G | Marginelidae | *Marginelid* sp. 1 | 1 | | | | | | 3 | | | | **4** |
| G | Marginelidae | *Marginelid* sp. 2 | | | 1 | | | 1 | 1 | 2 | 1 | | **6** |
| G | Mitridae | *Mitra rosocea* | 1 | | | 2 | | 7 | 1 | 2 | 1 | | **14** |
| G | Nassariidae | *Nassarius cf comptus* | | | | | 1 | | | | | | **1** |
| G | Nassariidae | *Nassarius macrophalus* | 1 | 2 | 3 | | | | | | | | **6** |
| G | Nassariidae | *Nassarius* sp. 3 | | | | | | | | 6 | | | **6** |
| G | Naticidae | *Natica fasciata* | 3 | 2 | | 1 | | | | 1 | 2 | | **9** |
| G | Naticidae | *Naticid* sp.1 | | | | | 1 | | | | | | **1** |
| G | Philinidae | *Philine* sp. 1 | 1 | | | 9 | 2 | | | 2 | 1 | 5 | **20** |
| G | Philinidae | *Philine* sp. 2 | 1 | | | 7 | 7 | | | 2 | | | **17** |
| G | Planaxidae | *Fossarus* sp. | | | 1 | | | | | | | | **1** |
| G | Pyramidellidae | *Turbonilla* sp. | 1 | 1 | 1 | | | 1 | 1 | 2 | | 1 | **8** |
| G | Pyramidellidae | *Pyramellid* sp. 2 | | 4 | | 1 | | | | 1 | | 1 | **7** |
| G | Pyramidellidae | *Pyramellid* sp. 3 | | | | | | | | 3 | | | **3** |
| G | Pyramidellidae | *Pyramellid* sp. 4 | | | | | | | | 2 | | | **2** |
| G | Retusidae | *Retusid* sp. 1 | | 1 | 2 | 1 | | | | | | | **4** |
| G | Ringiculidae | *Ringicula* sp. 1 | | | | 2 | | | | 13 | 2 | 3 | **20** |
| G | Ringiculidae | *Ringicula* sp. 2 | | | 1 | 5 | | | 4 | 17 | 5 | 1 | **33** |
| G | Rissoidae | *Rissoina c.f. sculptilis* | 2 | | | | | | | | 1 | | **3** |
| G | Rissoidae | *Rissoina sp.* | | 3 | 3 | | | 1 | | | | | **7** |
| G | Seraphsidae | *Terebellum terebellum* | 1 | | | | 1 | | | | | | **2** |
| G | Turridae | *Turricula* sp. 1 | 1 | | | 1 | 3 | | | 3 | | | **8** |
| G | Turridae | *Turrid* sp. 2 | 3 | 3 | | | | 3 | 1 | | 1 | | **11** |
| G | Turritelidae | *Turritelid* sp. 1 | | 6 | 2 | 7 | | | 2 | | | | **17** |
| G | Turritellidae | *Haustator cingulifer* | | | | | | | 11 | 3 | 2 | 3 | **19** |

**Table A1.1D**. The diversity of phylum **Echinodermata**: collected in the main study.

| Class | Family | Species or common name | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Asteroidea | Astropectinidae | *Astropecten* sp. | 2 | | | | | 2 | | | 1 | | **5** |
| Asteroidea | Goniasteridae | *Iconaster* sp. | | | | 1 | | | | | | | **1** |
| Crinoidea | Comasteridae | | | | | | 1 | | | | | | **1** |
| Echinoidea | | *Metalia* sp. | | 2 | 4 | | | | | 1 | | | **7** |
| Echinoidea | | Unknown Sand dollar | | | | 2 | | | | | | | **2** |
| Holothuroidea | | *Leptopentacta grisea* | 2 | 1 | 1 | 38 | 1 | 1 | 1 | | | | **45** |
| Holothuroidea | Synaptidae | | 2 | | | | 18 | 8 | 6 | 4 | 3 | 20 | **61** |
| Ophiuroidea | | Unknown brittlestar | | | | | | 2 | | | 2 | 1 | **5** |
| Ophiuroidea | Amphuridae | *Amphioplus laevis* | 301 | 316 | 197 | 319 | 176 | 195 | 305 | 232 | 379 | 315 | **2735** |
| Ophiuroidea | Ophiuridae | *Ophiura* sp. | 11 | 2 | 10 | 44 | 6 | | 6 | 4 | 14 | 3 | **100** |

**Table A1.1E.** The diversity of other phyla: collected in the main study from the sediments of the Kimberley coastal region.

| Phylum | Class | Order/ Family | Species or common name | Oct 06 | Jan 07 | May 07 | Sep 07 | Nov 07 | Feb 08 | Apr 08 | May 08 | Aug 08 | Nov 08 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chelicerates | Pycnogonida | Callipallenidae | *Propallene* sp. | 4 | | | | 1 | | 1 | | | 1 | **7** |
| Chordata | Osteichthyes | Anguillidae | *Anguilla* sp. | 1 | | | | 4 | | 2 | | 1 | | **8** |
| Chordata | Osteichthyes | Gobiidae | | 1 | 1 | 3 | | | | | 2 | | 2 | **9** |
| Chordata | Osteichthyes | Scorpaenidae | | 1 | | | | | | | 1 | | | **2** |
| Chordata | Osteichthyes | Platycephalidae | | | | | 1 | | | | | | | **1** |
| Cnidaria | Anthozoa | Ceriantharia | Tube dwelling anemone | 6 | | 2 | 4 | 1 | | | | | | **13** |
| Cnidaria | Anthozoa | Gorgonacea | Sea whip | | | | | 1 | 3 | | 4 | 3 | 5 | **16** |
| Cnidaria | Anthozoa | Gorgonacea | Sea fan | | | | 3 | | | | | | | **3** |
| Cnidaria | Anthozoa | Pennatulacea | Sea pen | 1 | 5 | | | | | 1 | | 2 | | **9** |
| Cnidaria | Anthozoa | Scleractinia | Stony coral | | | | 1 | | | | | | | **1** |
| Cnidaria | Hydrozoa | | Hydroid | | | | 1 | | | | | | | **1** |
| Cnidaria | Scyphozoa | | Jellyfish | | | | 8 | 5 | 1 | 1 | 22 | 4 | 9 | **50** |
| Echiura | | | Spoon worm | | | | 1 | | | | | | | **1** |
| Foraminifera | | | Forams | | 6 | 4 | 31 | 11 | 2 | 43 | 260 | 118 | 29 | **504** |
| Hemichordata | | | Acorn worm | 26 | 45 | 136 | 119 | | 5 | 13 | 12 | | 74 | **430** |
| Nemertea | | | Ribbon worm | 1 | | | | | | | 3 | | 33 | **37** |
| Platyhelminthes | | | Flat worm | 17 | 35 | 36 | 28 | | 8 | 8 | 5 | 2 | 1 | **140** |
| Porifera | | | Sponge | 30 | | | 4 | 6 | | 2 | | | | **42** |
| Priapulida | | | Priapulid worm | | | 1 | 11 | | | | | | | **12** |
| Sipuncula | | | Sipunculid | 16 | 8 | 5 | 19 | 2 | | 3 | 3 | 1 | 2 | **59** |
| Urochordata | Ascidiacea | | Tunicate | 6 | 1 | 12 | 6 | 2 | 2 | 8 | 3 | 7 | 3 | **50** |
| **ALL FAUNA** | | | **TOTALS** | **2735** | **2462** | **1734** | **2707** | **1673** | **1510** | **2296** | **2478** | **2331** | **2067** | **22015** |

**Table A1.2A.** The diversity of phylum **Annelida**: class **Polychaeta**, collected in the BACI study from the Kuri Bay region. B=Before, A=After, T=time.

| Order | Family | BT1 | BT2 | A1T1 | A1T2 | A2T1 | A2T2 | Total |
|---|---|---|---|---|---|---|---|---|
| Amphinomida | Amphinomidae | 2 | 11 | 4 | 4 | 6 | 11 | **38** |
| Capitellida | Capitellidae | 14 | 17 | 6 | 18 | 3 | 13 | **71** |
| Capitellida | Maldanidae | 2 | 11 | 6 | 29 | 1 | 8 | **57** |
| Eunicida | Eunicidae | 10 | 28 | 14 | 34 | 12 | 12 | **110** |
| Eunicida | Lumbrineridae | 2 | 13 | 11 | 8 | 1 | 6 | **41** |
| Eunicida | Oenonidae | 1 | 0 | 1 | 0 | 0 | 0 | **2** |
| Eunicida | Onuphidae | 3 | 6 | 7 | 1 | 4 | 1 | **22** |
| Flabelligerida | Flabelligeridae | 1 | 0 | 0 | 5 | 2 | 3 | **11** |
| Opheliida | Opheliidae | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| Orbiniida | Paraonidae | 5 | 7 | 5 | 7 | 1 | 2 | **27** |
| Phyllodocida | Acoetidae | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| Phyllodocida | Chrysopetalidae | 3 | 0 | 0 | 0 | 0 | 0 | **3** |
| Phyllodocida | Glyceridae | 0 | 3 | 2 | 7 | 1 | 0 | **13** |
| Phyllodocida | Goniadidae | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| Phyllodocida | Hesionidae | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| Phyllodocida | Nephtyidae | 10 | 4 | 2 | 6 | 5 | 3 | **30** |
| Phyllodocida | Nereididae | 6 | 3 | 0 | 1 | 2 | 6 | **18** |
| Phyllodocida | Phyllodocidae | 1 | 0 | 1 | 0 | 2 | 1 | **5** |
| Phyllodocida | Pilargidae | 1 | 3 | 1 | 2 | 1 | 1 | **9** |
| Phyllodocida | Polynoidae | 0 | 2 | 0 | 3 | 1 | 4 | **10** |
| Phyllodocida | Sigalionidae | 2 | 4 | 2 | 1 | 3 | 1 | **13** |
| Phyllodocida | Syllidae | 20 | 69 | 0 | 0 | 1 | 2 | **92** |
| Sabellida | Sabellidae | 3 | 3 | 3 | 3 | 1 | 4 | **17** |
| Spionida | Chaetopteridae | 1 | 0 | 1 | 1 | 1 | 1 | **5** |
| Spionida | Cirratulidae | 4 | 13 | 8 | 30 | 0 | 7 | **62** |
| Spionida | Magelonidae | 0 | 7 | 0 | 3 | 3 | 3 | **16** |
| Spionida | Poecilochaetidae | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Spionida | Spionidae | 3 | 6 | 7 | 7 | 6 | 14 | **43** |
| Sternaspida | Sternaspidae | 1 | 6 | 0 | 13 | 4 | 8 | **32** |
| Terebellida | Ampharetidae | 1 | 13 | 3 | 20 | 2 | 1 | **40** |
| Terebellida | Pectinariidae | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| Terebellida | Terebellidae | 23 | 30 | 13 | 50 | 24 | 48 | **188** |
| Terebellida | Trichobranchidae | 26 | 64 | 33 | 86 | 1 | 30 | **240** |

**Table A1.2B.** The diversity of phylum **Arthropoda**: subphylum **Crustacea**, class Malacostraca (M) or Ostracoda (O) collected in the BACI study (Orders: Amp= Amphipoda, Cum= Cumacea, Dec= Decapoda, Iso= Isopoda, Tan=Tanaidacea), (Sub-Infraorders: Brach=Brachyura, Carid= Caridea, Dend=Dendobranchiata). B=Before, A=After, T=time.

| Class | Order | Sub Infra | Family | Genus, Species or common name | BT1 | BT2 | A1T1 | A1T2 | A2T1 | A2T2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M | Amp | | Ampeliscidae | | 6 | 3 | 4 | 3 | 4 | 4 | **24** |
| M | Amp | | Aoridae | Aoridae sp. 1 | 2 | 1 | 2 | 0 | 0 | 0 | **5** |
| M | Amp | | Caprellidae | Phtisicinae | 0 | 1 | 0 | 3 | 0 | 0 | **4** |
| M | Amp | | Corophiidae | Corophiidae sp. 1 | 7 | 3 | 4 | 5 | 1 | 6 | **26** |
| M | Amp | | Corophiidae | Corophiidae sp. 2 | 4 | 0 | 5 | 10 | 0 | 0 | **19** |
| M | Amp | | Dexaminidae | Dexaminidae sp. 1 | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| M | Amp | | Leucathoidae | Leucathoidae sp. 1 | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| M | Amp | | Leucathoidae | Leucothoidae sp. 2 | 0 | 2 | 0 | 4 | 0 | 0 | **6** |
| M | Amp | | Leucathoidae | Leucothoidae sp. 3 | 0 | 1 | 0 | 0 | 1 | 0 | **2** |
| M | Amp | | Liljeborgiidae | Liljeborgiidae sp. 2 | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| M | Amp | | Lysianassidae | *Acidostoma sp.* | 4 | 1 | 0 | 0 | 1 | 1 | **7** |
| M | Amp | | Melitidae | *Malacoota chandaniae* | 0 | 1 | 0 | 1 | 0 | 1 | **3** |
| M | Amp | | Phoxo-cephalidae | Phoxocephalid sp. 1 | 0 | 2 | 0 | 1 | 0 | 2 | **5** |
| M | Amp | | Podoceridae | Podoceridae sp. 1 | 1 | 1 | 0 | 1 | 2 | 5 | **10** |
| M | Amp | | Stenothoidae | Stenothoidae sp. 1 | 0 | 1 | 0 | 1 | 0 | 2 | **4** |
| M | Cum | | | Cumacea | 0 | 4 | 1 | 3 | 2 | 3 | **13** |
| M | Dec | Brach | Ebaliinae | Ebaliinae sp. 1 | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| M | Dec | Brach | Ebaliinae | Ebaliinae sp. 2 | 1 | 0 | 0 | 1 | 0 | 0 | **2** |
| M | Dec | Brach | Goneplacidae | Goneplacidae sp. 1 | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| M | Dec | Brach | Goneplacidae | Goneplacidae sp. 2 | 0 | 3 | 1 | 0 | 0 | 0 | **4** |
| M | Dec | Brach | Hypolytidae | *Alopes* sp. 1 | 0 | 2 | 1 | 0 | 0 | 0 | **3** |
| M | Dec | Brach | Leusosiidae | | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 1 | 1 | 7 | 0 | 1 | 3 | 0 | **12** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 2 | 4 | 0 | 0 | 4 | 1 | 0 | **9** |
| M | Dec | Brach | Pilumnidae | Pilumnidae sp. 3 | 4 | 4 | 0 | 0 | 1 | 73 | **82** |
| M | Dec | Brach | Pilumnidae | Rhizopinae | 8 | 15 | 15 | 18 | 7 | 13 | **76** |
| M | Dec | Brach | Pilumnidae | Rhizopinae *Ceratoplax* sp. | 1 | 0 | 0 | 0 | 1 | 0 | **2** |
| M | Dec | Brach | Pilumnidae | *Cryptolutea arafurensis* | 1 | 2 | 0 | 0 | 0 | 0 | **3** |
| M | Dec | Brach | Pinnotheridae | | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| M | Dec | Brach | Portunidae | *Thalamita* sp. | 1 | 0 | 1 | 1 | 1 | 0 | **4** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 1 | 4 | 2 | 3 | 2 | 3 | 0 | **14** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 2 | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| M | Dec | Carid | Alpheidae | *Alpheus* sp. 3 | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| M | Dec | Carid | Axiidae | *Paraxopsis dianae* | 0 | 1 | 4 | 2 | 2 | 13 | **22** |
| M | Dec | Carid | Crangonidae | *Philocheras* sp. | 3 | 1 | 0 | 0 | 0 | 3 | **7** |
| M | Dec | Carid | Ogyrididae | *Ogyrides* sp. | 15 | 7 | 14 | 5 | 10 | 6 | **57** |
| M | Dec | Carid | Palaemonidae | | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| M | Dec | Carid | Pasiphaeidae | *Leptochela sydniensis* | 12 | 3 | 7 | 4 | 8 | 5 | **39** |

**Table A1.2B** continued. The diversity of **Arthropoda**: subphylum **Crustacea**.

| Class | Order | Sub Infra | Family | Genus, Species or common name | BT1 | BT2 | A1T1 | A1T2 | A2T1 | A2T2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M | Dec | Carid | Processidae | *Processa longirostris* | 2 | 0 | 1 | 0 | 3 | 1 | **7** |
| M | Dec | Dend | Luciferidae | | 7 | 1 | 14 | 5 | 2 | 0 | **29** |
| M | Dec | Dend | Penaeidae | | 3 | 0 | 2 | 3 | 0 | 0 | **8** |
| M | Dec | Dend | Sergestidae | *Sergia* sp. | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| M | Iso | | Anthuridae | *Amakusanthura* sp. | 15 | 4 | 1 | 1 | 0 | 3 | **24** |
| M | Iso | | Arcturidae | *Neastacilla* sp. | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| M | Iso | | Cirolanidae | | 0 | 2 | 0 | 0 | 0 | 0 | **2** |
| M | Mysidacea | | | Mysid | 0 | 3 | 1 | 0 | 0 | 0 | **4** |
| M | Nebaliacea | | | *Nebalia* sp. | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| M | Stomapoda | | | Stomatopod | 1 | 0 | 1 | 2 | 1 | 1 | **6** |
| M | Tan | Tanaidomorpha | | *Leptochelia* or *Bathytanais* sp. | 1 | 2 | 0 | 2 | 1 | 0 | **6** |
| M | Tan | Apseudomorpha | | *Saltipedis* or *Whiteleggia* sp. | 216 | 28 | 21 | 20 | 15 | 21 | **321** |
| O | | | | Ostracoda sp. 1 | 2 | 0 | 3 | 6 | 1 | 0 | **12** |

**Table A1.2C.** The diversity of phylum **Mollusca**: class **Bivalva (B) or Gastropoda (G)**, collected in the BACI study from Kuri Bay. B=Before, A=After, T=time.

| Class | Order | Family | Genus or Species | BT1 | BT2 | A1T1 | A1T2 | A2T1 | A2T2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| B | Arcoida | Arcidae | *Baritia barbitia* | 3 | 0 | 0 | 0 | 0 | 0 | **3** |
| B | Myoida | Corbulidae | *Anisocorbula macgillivrayi* | 3 | 2 | 6 | 5 | 12 | 0 | **28** |
| B | Myoida | Corbulidae | *Serracorbula solidula* | 34 | 9 | 5 | 11 | 3 | 3 | **65** |
| B | Mytiloida | Mytelidae | *Arcuatula* sp. | 1 | 0 | 0 | 0 | 0 | 2 | **3** |
| B | Nuculoida | Nuculanidae | *Nuculana c.f. darwini* | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| B | Nuculoida | Nuculidae | *Leionucula cumingi* | 2 | 0 | 0 | 0 | 0 | 1 | **3** |
| B | Nuculoida | Nuculidae | *Leionucula definata* | 0 | 7 | 0 | 0 | 0 | 1 | **8** |
| B | Nuculoida | Nuculidae | *Leionucula obliqua* | 1 | 3 | 0 | 0 | 1 | 2 | **7** |
| B | Nuculoida | Nuculidae | *Nucula* sp. 2 | 0 | 0 | 0 | 4 | 0 | 0 | **4** |
| B | Nuculoida | Nuculidae | *Nuculid* sp. 1 | 0 | 0 | 1 | 1 | 0 | 0 | **2** |
| B | Pholadomyoida | Myochamidae | *Myadora* sp. | 3 | 0 | 0 | 0 | 0 | 0 | **3** |
| B | Solemyoida | Solemyidae | *Theora fragilis* | 2 | 2 | 0 | 1 | 13 | 1 | **19** |
| B | Veneroida | Galeom-matidae | *Curvemysella c.f. paula* | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| B | Veneroida | Lucinidae | *Anodontia* sp. | 4 | 0 | 0 | 1 | 1 | 1 | **7** |
| B | Veneroida | Lucinidae | *Cardiolucina eucosmia* | 6 | 1 | 6 | 10 | 1 | 3 | **27** |
| B | Veneroida | Lucinidae | *Lucine* sp. 1 | 5 | 6 | 6 | 8 | 2 | 3 | **30** |
| B | Veneroida | Pharidae | *Nucula torresi* | 4 | 0 | 0 | 0 | 0 | 0 | **4** |
| B | Veneroida | Semelidae | *Sinonovacula constricta* | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| B | Veneroida | Tellinidae | *Macoma* sp. 1 | 0 | 1 | 2 | 2 | 0 | 1 | **6** |
| B | Veneroida | Tellinidae | *Macoma* sp. 2 | 16 | 10 | 2 | 21 | 2 | 6 | **57** |
| B | Veneroida | Tellinidae | *Solenmyid* sp. 1 | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| B | Veneroida | Veneridae | *Pitar* sp. | 8 | 4 | 4 | 7 | 0 | 8 | **31** |
| G | | Columbellidae | *Zafra* sp. 1 | 1 | 1 | 0 | 2 | 0 | 0 | **4** |
| G | | Cerithiidae | *Cerithium* sp. 2 | 3 | 0 | 0 | 0 | 0 | 0 | **3** |
| G | | Cylichnidae | *Cylichna* sp. 1 | 2 | 1 | 0 | 4 | 0 | 3 | **10** |
| G | | Turritellidae | *Turritelid* sp. | 4 | 0 | 0 | 2 | 0 | 0 | **6** |
| G | | Philinidae | *Philine* sp. 1 | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| G | | Philinidae | *Philine* sp. 2 | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| G | | Ringiculidae | *Ringicula* sp. 1 | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| G | | Turritelidae | *Haustator cingulifer* | 0 | 1 | 0 | 0 | 0 | 1 | **2** |

**Table A1.2D**. The diversity of other phyla: collected in the BACI study from the sediments of the Kuri Bay region. B=Before, A=After, T=time.

| Phylum | Class | Order | Family | Species or common name | BT1 | BT2 | A1T1 | A1T2 | A2T1 | A2T2 | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chelicerates | Pycnogonida | | Callipallenidae | *Propallene* sp. | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Chordata | Osteichthyes | | Gobiidae | | 1 | 0 | 1 | 0 | 0 | 0 | **2** |
| Chordata | Osteichthyes | | Blenniidae | | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| Chordata | Osteichthyes | | Platycephalidae | | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| Cnidaria | Anthozoa | Gorgonacea | | Sea whip | 0 | 0 | 0 | 0 | 1 | 2 | **3** |
| Cnidaria | Anthozoa | Scleractinia | | Stoney coral | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| Echinodermata | Echinoidea | | | *Metalia* sp. | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| Echinodermata | Holothuroidea | | | *Leptopentacta grisea* | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| Echinodermata | Ophiuroidea | | Amphuridae | *Amphioplus laevis* | 27 | 13 | 7 | 2 | 9 | 12 | **70** |
| Echinodermata | Ophiuroidea | | Ophiuridae | *Ophiura* sp. | 0 | 8 | 5 | 19 | 2 | 3 | **37** |
| Foraminifera | | | | Forams | 2 | 0 | 0 | 7 | 0 | 0 | **9** |
| Hemichordata | | | | Acorn worm | 7 | 0 | 47 | 92 | 9 | 59 | **214** |
| Platyhelminthes | | | | Flat Worm | 1 | 0 | 8 | 17 | 1 | 0 | **27** |
| Porifera | | | | Sponge | 0 | 0 | 0 | 4 | 0 | 0 | **4** |
| Sipuncula | | | | Sipunculid | 6 | 9 | 5 | 11 | 1 | 0 | **32** |
| Urochordata | | | | Tunicate | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| **ALL FAUNA** | | | | **TOTALS** | **620** | **513** | **347** | **696** | **220** | **470** | **2880** |

## APPENDIX TWO: ANOVAS COMPARING ALL BAYS IN MAIN STUDY

**Table A2.1.** ANOVA results comparing the number of benthic macrofauna taxa in the soft sediments at ten different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 2214.0 | 246.0 | 8.37 | | | Loc(Bay) x Time |
| Bays | 2 | 2109.0 | 1054.5 | 5.04 | | | Loc(Bay) |
| Time x Bays | 18 | 1957.4 | 108.7 | 3.70 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 2509.5 | 209.1 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 230.4 | 76.8 | **0.30** | **0.822** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 2279.1 | 253.2 | 8.20 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 856.3 | 28.5 | 1.97 | 0.003 | | Residuals |
| Sites (Farms) | (6) | 115.3 | 19.2 | 0.62 | 0.711 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 741.0 | 30.9 | 1.99 | 0.005 | | |
| Locations(Bay) x Time | 108 | 3174.8 | 29.4 | 2.03 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (27) | 823.1 | 30.5 | **1.05** | **0.418** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (81) | 2351.7 | 29.0 | 1.07 | 0.0002 | | |
| Sites(Loc(Bay)) x Time | 270 | 3904.8 | 14.5 | 1.27 | 0.006 | Yes | Residuals |
| Sites (Farms) x Time | (54) | 554.5 | 10.3 | 0.66 | 0.963 | NS | Sites(Refs) x Time |
| Sites (Refs) x Time | (216) | 3350.3 | 15.5 | | | | |
| Residuals | 900 | 10265.3 | 11.4 | | | | |
| Total | 1349 | 26991.2 | | | | | |

**Table A2.2.** ANOVA results comparing the number of benthic macrofauna individuals in the soft sediments at ten different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 66.39 | 7.38 | 5.57 | | | Loc(Bay) x Time |
| Bays | 2 | 16.81 | 8.40 | 0.96 | | | Loc(Bay) |
| Time x Bays | 18 | 65.46 | 3.64 | 2.75 | 0.0006 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 104.86 | 8.74 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 29.99 | 10.00 | **1.20** | **0.364** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 74.87 | 8.32 | 5.24 | 0.0005 | | |
| Sites (Loc(Bays)) | 30 | 42.29 | 1.41 | 3.34 | <0.0001 | | Residuals |
| Sites (Farms) | (6) | 4.21 | 0.70 | 0.44 | 0.843 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 38.09 | 1.59 | 3.57 | <0.0001 | | |
| Locations(Bay) x Time | 108 | 143.02 | 1.32 | 3.13 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (27) | 32.37 | 1.20 | **0.88** | **0.639** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (81) | 110.64 | 1.37 | 3.07 | <0.0001 | | |
| Sites(Loc(Bay)) x Time | 270 | 114.13 | 0.42 | 1.53 | <0.0001 | Yes | Residuals |
| Sites (Farms) x Time | (54) | 18.11 | 0.34 | 0.75 | 0.890 | NS | Sites(Refs) x Time |
| Sites (Refs) x Time | (216) | 96.02 | 0.44 | | | | |
| Residuals | 900 | 248.14 | 0.28 | | | | |
| Total | 1349 | 801.09 | | | | | |

**Table A2.3.** ANOVA results comparing percentage of Total Organic Matter (TOM) in the soft sediments at nine different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 149.46 | 18.68 | 4.49 | | | Loc(Bay) x Time |
| Bays | 2 | 4809.94 | 2404.97 | 54.80 | | | Loc(Bay) |
| Time xBays | 16 | 228.90 | 14.31 | 3.44 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 526.61 | 43.88 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 79.00 | 26.33 | **0.53** | **0.673** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 447.61 | 49.73 | 12.85 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 131.97 | 4.40 | 4.42 | <0.0001 | | Residuals |
| Sites (Farms) | (6) | 39.06 | 6.51 | 1.68 | 0.169 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 92.91 | 3.87 | 4.30 | <0.0001 | | |
| Loc(Bay) x Time | 96 | 399.14 | 4.16 | 4.18 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (24) | 97.20 | 4.05 | **0.97** | **0.519** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (72) | 301.94 | 4.19 | 4.66 | <0.0001 | | |
| Sites(Loc(Bay)) x Time | 240 | 238.62 | 0.99 | 2.53 | <0.0001 | | Residuals |
| Sites (Farms) x Time | (48) | 65.85 | 1.37 | 1.52 | 0.025 | Yes | Sites(Refs) x Time |
| Sites (Refs) x Time | (192) | 172.77 | 0.90 | | | | |
| Residuals | 810 | 318.55 | 0.39 | | | | |
| Total | 1214 | 6803.17 | | | | | |

**Table A2.4.** ANOVA results comparing percentage of nitrogen in the soft sediments at nine different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 0.4609 | 0.0576 | 8.80 | | | Loc(Bay) x Time |
| Bays | 2 | 2.0636 | 1.0318 | 157.53 | | | Loc(Bay) |
| Time x Bays | 16 | 0.1048 | 0.0066 | 8.34 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 0.1086 | 0.0091 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 0.0267 | 0.0089 | **0.98** | **0.445** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 0.0819 | 0.0091 | 11.74 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 0.0192 | 0.0006 | 2.84 | <0.0001 | | Residuals |
| Sites (Farms) | (6) | 0.0006 | 0.0001 | 0.03 | 0.999 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 0.0186 | 0.0008 | 3.36 | <0.0001 | | |
| Loc (Bay) x Time | 96 | 0.0754 | 0.0008 | 3.49 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (24) | 0.0127 | 0.0005 | **0.61** | **0.914** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (72) | 0.0627 | 0.0009 | 3.77 | <0.0001 | | |
| Sites (Loc(Bay)) x Time | 240 | 0.0540 | 0.0002 | 3.76 | <0.0001 | | Residuals |
| Sites (Farms) x Time | (48) | 0.01 | 0.0000 | 0.88 | 0.701 | NS | Sites(Refs) x Time |
| Sites (Refs) x Time | (192) | 0.04 | 0.0000 | | | | |
| Residuals | 810 | 0.0646 | 0.0001 | | | | |
| Total | 1214 | 2.9512 | | | | | |

**Table A2.5.** ANOVA results comparing percentage of carbon in the soft sediments at nine different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 106.53 | 13.32 | 9.32 | | | Loc(Bay) x Time |
| Bays | 2 | 727.15 | 363.5 | 28.71 | | | Loc(Bay) |
| Time x Bays | 16 | 114.59 | 7.16 | 5.01 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 151.98 | 12.67 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 34.51 | 11.50 | **0.88** | **0.487** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 117.47 | 13.05 | 10.86 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 30.02 | 1.00 | 4.11 | <0.0001 | | Residuals |
| Sites (Farms) | (6) | 1.16 | 0.19 | 0.16 | 0.985 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 28.86 | 1.20 | 4.19 | <0.0001 | | |
| Locations(Bay) x Time | 96 | 137.12 | 1.43 | 5.87 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (24) | 34.79 | 1.45 | **1.02** | **0.454** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (72) | 102.33 | 1.42 | 4.96 | <0.0001 | | |
| Sites (Loc(Bay)) x Time | 240 | 58.38 | 0.24 | 2.81 | <0.0001 | | Residuals |
| Sites (Farms) x Time | (48) | 3.32 | 0.07 | 0.24 | 0.999 | NS | Sites(Refs) x Time |
| Sites (Refs) x Time | (192) | 55.06 | 0.29 | | | | |
| Residuals | 810 | 70.10 | 0.09 | | | | |
| Total | 1214 | 1395.87 | | | | | |

**Table A2.6.** ANOVA results comparing the phosphorus content in the soft sediments at eight different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Var. | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 7 | 407829 | 58261 | 2.20 | | | Loc(Bay) x Time |
| Bays | 2 | 4999187 | 2499593 | 13.40 | | | Loc(Bay) |
| Time x Bays | 14 | 1888524 | 134894 | 5.11 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 2239109 | 186592 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 399255 | 133085 | **0.65** | **0.602** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 1839854 | 204428 | 27.20 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 221975 | 7399 | 1.24 | 0.193 | | Residuals |
| Sites (Farms) | (6) | 41602 | 6933 | 0.92 | 0.496 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 180372 | 7515 | 1.17 | 0.275 | | |
| Locations(Bay) x Ti | 84 | 2219521 | 26422 | 4.43 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Ti** | (21) | 591633 | 28173 | **1.09** | **0.381** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Ti | (63) | 1627887 | 25839 | 4.03 | <0.0001 | | |
| Sites(Loc(Bay))x Ti | 210 | 1253383 | 5968 | 1.54 | <0.0001 | | Residuals |
| Sites(Farms) x Ti | (42) | 175848 | 4186 | 0.65 | 0.947 | NS | Sites (Refs) x Time |
| Sites (Refs) x Time | (168) | 1077534 | 6413 | | | | |
| Residuals | 720 | 2784775 | 3867 | | | | |
| Total | 1079 | 16014306 | | | | | |

**Table A2.7.** ANOVA results comparing percentage of carbonates in the soft sediments at nine different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 494.50 | 61.81 | 5.13 | | | Loc(Bay) x Time |
| Bays | 2 | 34896.20 | 17448.1 | 81.42 | | | Loc(Bay) |
| Time x Bays | 16 | 575.76 | 35.98 | 2.99 | 0.0005 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 2571.58 | 214.30 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 374.38 | 124.79 | **0.51** | **0.684** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 2197.20 | 244.13 | 9.78 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 618.57 | 20.62 | 4.58 | <0.0001 | | Residuals |
| Sites (Farms) | (6) | 19.31 | 3.22 | 0.13 | 0.991 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 599.26 | 24.97 | 5.16 | <0.0001 | | |
| Locations(Bay) x Time | 96 | 1156.18 | 12.04 | 2.68 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Time** | (24) | 183.83 | 7.66 | **0.57** | **0.940** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (72) | 972.34 | 13.50 | 2.79 | <0.0001 | | |
| Sites (Loc(Bay)) x Time | 240 | 1079.53 | 4.50 | 2.77 | <0.0001 | | Residuals |
| Sites (Farms) x Time | (48) | 150.61 | 3.14 | 0.65 | 0.961 | NS | Sites (Refs) x Time |
| Sites (Refs) x Time | (192) | 928.92 | 4.84 | | | | |
| Residuals | 810 | 1317.27 | 1.63 | | | | |
| Total | 1214 | 42709.58 | | | | | |

**Table A2.8.** ANOVA results comparing the redox potential in the soft sediments at seven different sampling times, in three bays, among three farms and twelve reference locations and with three sites nested in each of these locations. NS = No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 6 | 732307.9 | 122051.3 | 19.95 | | | Loc(Bay) x Time |
| Bays | 2 | 111832.5 | 55916.2 | 0.66 | | | Loc(Bay) |
| Time x Bays | 12 | 452044.6 | 37670.4 | 6.16 | <0.0001 | | Loc(Bay) x Time |
| Locations (Bays) | 12 | 1015392.4 | 84616.0 | | | | Sites (Loc(Bays)) |
| **Farms vs. Refs** | (3) | 317775.5 | 105925.2 | **1.37** | **0.314** | NS | Loc (Bays); Refs |
| Among Refs | (9) | 697616.9 | 77512.9 | 22.29 | <0.0001 | | |
| Sites (Loc(Bays)) | 30 | 96846.8 | 3228.2 | 2.03 | 0.003 | | Residuals |
| Sites (Farms) | (6) | 13402.4 | 2233.7 | 0.64 | 0.695 | NS | Sites (Refs) |
| Sites (Refs) | (24) | 83444.4 | 3476.9 | 2.19 | 0.002 | | |
| Locations(Bay) x Time | 72 | 440417.4 | 6116.9 | 3.85 | <0.0001 | | Sites(Loc(Bay))x Ti |
| **Farm vs. Refs x Ti** | (18) | 56009.2 | 3111.6 | **0.44** | **0.972** | NS | Loc(Bay);Refs x Ti |
| Among Refs x Time | (54) | 384408.2 | 7118.7 | 4.48 | <0.0001 | | |
| Sites (Loc(Bay)) x Ti | 180 | 266250.0 | 1479.2 | 1.62 | <0.0001 | | Residuals |
| Sites (Farms) x Ti | (36) | 37668.8 | 1046.4 | 0.66 | 0.927 | NS | Sites(Refs) x Time |
| Sites (Refs) x Ti | (144) | 228581.3 | 1587.4 | | | | |
| Residuals | 640 | 574810.6 | 898.1 | | | | |
| Total | 944 | 3721821.5 | | | | | |

## APPENDIX THREE: ANOVAS COMPARING WIHTIN EACH BAY (RUN SEPARATELY FOR EACH BAY)

### CYGNET BAY ANOVAS

**Table A3.1.** ANOVA results comparing the number of benthic macrofauna taxa in the soft sediments at Cygnet Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 826.98 | 91.89 | | | | Loc x Time |
| Locations | 4 | 1221.66 | 305.41 | | | | Sites (Loc) |
| **Farms vs. Refs** | (1) | 57.96 | 57.96 | **0.15** | **0.725** | NS | Loc; Refs |
| Among Refs | (3) | 1163.70 | 387.90 | 14.61 | 0.001 | | |
| Sites (Loc(Bay)) | 10 | 219.29 | 21.93 | | | | Residuals |
| Sites (Farms) | (2) | 6.96 | 3.48 | 0.13 | 0.879 | NS | Sites (Refs) |
| Sites (Refs) | (8) | 212.33 | 26.54 | 1.78 | 0.095 | | |
| Locations x Time | 36 | 1403.90 | 39.00 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | (9) | 398.51 | 44.28 | **1.19** | **0.341** | NS | Loc;Refs x Ti |
| Among Refs x Time | (27) | 1005.39 | 37.24 | 2.50 | 0.001 | | |
| Sites(Loc) x Time | 90 | 1325.82 | 14.73 | | | | Residuals |
| Sites (Farms) x Time | (18) | 253.93 | 14.11 | 0.95 | 0.527 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | (72) | 1071.89 | 14.89 | 1.05 | 0.387 | | |
| Residuals | 300 | 4265.33 | 14.22 | | | | |
| Total | 449 | 9262.98 | | | | | |

**Table A3.2.** ANOVA results comparing the number of benthic macrofauna individuals in the soft sediments at Cygnet Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 20.72 | 2.30 | | | | Loc x Time |
| Locations | 4 | 21.59 | 5.40 | | | | Sites (Loc) |
| **Farms vs. Refs** | (1) | 0.83 | 0.83 | **0.12** | **0.752** | NS | Loc; Refs |
| Among Refs | (3) | 20.76 | 6.92 | 13.70 | 0.002 | | |
| Sites (Loc(Bay)) | 10 | 4.07 | 0.41 | | | | Residuals |
| Sites (Farms) | (2) | 0.02 | 0.01 | 0.02 | 0.976 | NS | Sites (Refs) |
| Sites (Refs) | (8) | 4.04 | 0.51 | 1.85 | 0.085 | | |
| Locations x Time | 36 | 43.74 | 1.21 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | (9) | 13.71 | 1.52 | **1.37** | **0.250** | NS | Loc;Refs x Ti |
| Among Refs x Time | (27) | 30.02 | 1.11 | 4.04 | 0.000 | | |
| Sites(Loc) x Time | 90 | 26.09 | 0.29 | | | | Residuals |
| Sites (Farms) x Time | (18) | 6.25 | 0.35 | 1.26 | 0.240 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | (72) | 19.84 | 0.28 | 1.08 | 0.323 | | |
| Residuals | 300 | 76.47 | 0.25 | | | | |
| Total | 449 | 192.67 | | | | | |

**Table A3.3.** ANOVA results comparing the %TOM in the soft sediments at Cygnet Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 0.40 | 0.05 | | | | Loc x Time |
| Locations | 4 | 3.67 | 0.92 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.02 | 0.02 | **0.02** | **0.900** | NS | Loc; Refs |
| Among Refs | 3 | 3.64 | 1.21 | 24.95 | <0.000 | 0.000 | |
| Sites (Loc(Bay)) | 10 | 0.72 | 0.07 | | | | Residuals |
| Sites (Farms) | 2 | 0.33 | 0.17 | 3.40 | 0.085 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.39 | 0.05 | 4.48 | <0.000 | | |
| Locations x Time | 32 | 1.10 | 0.03 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.22 | 0.03 | **0.74** | **0.659** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 0.88 | 0.04 | 3.38 | <0.000 | | |
| Sites(Loc) x Time | 80 | 1.08 | 0.01 | | | | Residuals |
| Sites (Farms) x Time | 16 | 0.39 | 0.02 | 2.24 | 0.012 | SIG | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.70 | 0.01 | 1.56 | 0.008 | | |
| Residuals | 270 | 1.88 | 0.01 | | | | |
| Total | 404 | 8.85 | 0.02 | | | | |

**Table A3.4.** ANOVA results comparing the %N in the soft sediments at Cygnet Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 0.0981 | 0.0123 | | | | Loc x Time |
| Locations | 4 | 0.0078 | 0.0020 | 12.19 | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.0001 | 0.0001 | **0.04** | **0.856** | NS | Loc; Refs |
| Among Refs | 3 | 0.0077 | 0.0026 | 14.67 | 0.001 | | |
| Sites (Loc(Bay)) | 10 | 0.0016 | 0.0002 | 4.80 | | | Residuals |
| Sites (Farms) | 2 | 0.0002 | 0.0001 | 0.57 | 0.586 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.0014 | 0.0002 | 0.97 | 0.464 | | |
| Locations x Time | 32 | 0.0120 | 0.0004 | 1.97 | 0.008 | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.0033 | 0.0004 | **1.14** | **0.375** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 0.0087 | 0.0004 | 2.02 | 0.014 | | |
| Sites(Loc) x Time | 80 | 0.0152 | 0.0002 | 5.70 | <0.000 | | Residuals |
| Sites (Farms) x Time | 16 | 0.0037 | 0.0002 | 1.29 | 0.233 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.0115 | 0.0002 | 5.39 | <0.000 | | |
| Residuals | 270 | 0.0090 | 0.0000 | | | | |
| Total | 404 | | | | | | |

**Table A3.5.** ANOVA results comparing the %C in the soft sediments at Cygnet Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 1.67 | 0.21 | | | | Loc x Time |
| Locations | 4 | 1.83 | 0.46 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.03 | 0.03 | **0.05** | **0.843** | NS | Loc; Refs |
| Among Refs | 3 | 1.80 | 0.60 | 112.2 | <0.000 | | |
| Sites (Loc(Bay)) | 10 | 0.06 | 0.01 | | | | Residuals |
| Sites (Farms) | 2 | 0.02 | 0.01 | 1.47 | 0.286 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.04 | 0.01 | 1.25 | 0.288 | | |
| Locations x Time | 32 | 0.22 | 0.01 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.09 | 0.01 | **1.93** | **0.101** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 0.14 | 0.01 | 1.33 | 0.183 | | |
| Sites(Loc) x Time | 80 | 0.38 | 0.00 | | | | Residuals |
| Sites (Farms) x Time | 16 | 0.10 | 0.01 | 1.51 | 0.126 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.27 | 0.00 | 2.18 | <0.000 | | |
| Residuals | 270 | 0.53 | 0.00 | | | | |
| Total | 404 | 4.68 | 0.01 | | | | |

**Table A3.6.** ANOVA results comparing the P in the soft sediments at Cygnet Bay over eight different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 7 | 6.34 | 0.91 | | | | Loc x Time |
| Locations | 4 | 8.39 | 2.10 | 73.97 | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.62 | 0.62 | **0.24** | **0.658** | NS | Loc; Refs |
| Among Refs | 3 | 7.77 | 2.59 | 81.59 | <0.000 | | |
| Sites (Loc(Bay)) | 10 | 0.28 | 0.03 | 1.39 | 0.186 | | Residuals |
| Sites (Farms) | 2 | 0.03 | 0.01 | 0.47 | 0.643 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.25 | 0.03 | 0.62 | 0.759 | | |
| Locations x Time | 28 | 2.07 | 0.07 | 1.60 | 0.058 | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 7 | 0.36 | 0.05 | **0.63** | **0.723** | NS | Loc;Refs x Ti |
| Among Refs x Time | 21 | 1.71 | 0.08 | 1.58 | 0.087 | | |
| Sites(Loc) x Time | 70 | 3.23 | 0.05 | 2.26 | | | Residuals |
| Sites (Farms) x Time | 14 | 0.36 | 0.03 | 0.49 | 0.926 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 56 | 2.88 | 0.05 | 2.52 | <0.000 | | |
| Residuals | 240 | 4.90 | 0.02 | | | | |
| Total | 359 | 25.23 | 0.07 | | | | |

**Table A3.7.** ANOVA results comparing the %Carbonates in the soft sediments at Cygnet Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 1.22 | 0.15 | | | | Loc x Time |
| Locations | 4 | 2.81 | 0.70 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.12 | 0.12 | **0.14** | **0.735** | NS | Loc; Refs |
| Among Refs | 3 | 2.69 | 0.90 | 155.4 | <0.000 | | |
| Sites (Loc(Bay)) | 10 | 0.06 | 0.01 | | | | Residuals |
| Sites (Farms) | 2 | 0.01 | 0.01 | 1.16 | 0.360 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.05 | 0.01 | 1.39 | 0.217 | | |
| Locations x Time | 32 | 0.17 | 0.01 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.05 | 0.01 | **1.86** | **0.078** | NS | Site(Loc) x Time |
| Among Refs x Time | 24 | 0.11 | 0.00 | 1.13 | 0.342 | | |
| Sites(Loc) x Time | 80 | 0.29 | 0.00 | | | | Residuals |
| Sites (Farms) x Time | 16 | 0.02 | 0.00 | 0.31 | 0.994 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.27 | 0.00 | 2.17 | <0.000 | | |
| Residuals | 270 | 0.52 | 0.00 | | | | |
| Total | 404 | 5.06 | 0.01 | | | | |

**Table A3.8.** ANOVA results comparing the Redox potential of the soft sediments at Cygnet Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 735713 | 91964 | | | | Loc x Time |
| Locations | 4 | 665943 | 166486 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 115446 | 115446 | **0.63** | **0.486** | NS | Loc; Refs |
| Among Refs | 3 | 550497 | 183499 | 50.09 | <0.000 | | |
| Sites (Loc(Bay)) | 10 | 48571 | 4857 | | | | Residuals |
| Sites (Farms) | 2 | 19262 | 9631 | 2.63 | 0.133 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 29309 | 3664 | 2.91 | 0.008 | | |
| Locations x Time | 32 | 161084 | 5034 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 43414 | 5427 | **1.11** | **0.393** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 117670 | 4903 | 3.89 | <0.000 | | |
| Sites(Loc) x Time | 80 | 97405 | 1218 | | | | Residuals |
| Sites (Farms) x Time | 16 | 16844 | 1053 | 0.84 | 0.641 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 80561 | 1259 | 2.06 | <0.000 | | |
| Residuals | 270 | 164835 | 611 | | | | |
| Total | 404 | 1873551 | 4638 | | | | |

## PORT GEORGE ANOVAS

**Table A3.9.** ANOVA results comparing the number of benthic macrofauna taxa in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 63.28 | 7.03 | | | | Loc x Time |
| Locations | 4 | 14.09 | 3.52 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.01 | 0.01 | **0.00** | **0.972** | NS | Loc; Refs |
| Among Refs | 3 | 14.09 | 4.70 | 9.80 | 0.005 | | |
| Sites (Loc(Bay)) | 10 | 5.73 | 0.57 | | | | Residuals |
| Sites (Farms) | 2 | 1.90 | 0.95 | 1.98 | 0.200 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 3.83 | 0.48 | 1.03 | 0.420 | | |
| Locations x Time | 36 | 37.77 | 1.05 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 8.48 | 0.94 | **0.87** | **0.563** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 29.29 | 1.08 | 2.34 | 0.002 | | |
| Sites(Loc) x Time | 90 | 37.23 | 0.41 | | | | Residuals |
| Sites (Farms) x Time | 18 | 3.83 | 0.21 | 0.46 | 0.967 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 33.40 | 0.46 | 1.36 | 0.040 | | |
| Residuals | 300 | 102.10 | 0.34 | | | | |
| Total | 449 | 260.20 | | | | | |

**Table A3.10.** ANOVA results comparing the number of benthic macrofauna individuals in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 41.80 | 4.64 | | | | Loc x Time |
| Locations | 4 | 10.99 | 2.75 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.05 | 0.05 | **0.01** | **0.912** | NS | Loc; Refs |
| Among Refs | 3 | 10.93 | 3.64 | 11.27 | 0.003 | | |
| Sites (Loc(Bay)) | 10 | 4.19 | 0.42 | | | | Residuals |
| Sites (Farms) | 2 | 1.60 | 0.80 | 2.47 | 0.146 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 2.59 | 0.32 | 0.82 | 0.587 | | |
| Locations x Time | 36 | 43.30 | 1.20 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 7.72 | 0.86 | **0.65** | **0.744** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 35.58 | 1.32 | 3.34 | <0.000 | | |
| Sites(Loc) x Time | 90 | 31.55 | 0.35 | | | | Residuals |
| Sites (Farms) x Time | 18 | 3.16 | 0.18 | 0.45 | 0.972 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 28.39 | 0.39 | 1.52 | 0.008 | | |
| Residuals | 300 | 77.60 | 0.26 | | | | |
| Total | 449 | 209.43 | | | | | |

**Table A3.11.** ANOVA results comparing the %TOM in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 203.78 | 22.64 | | | | Loc x Time |
| Locations | 4 | 87.07 | 21.77 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 36.25 | 36.25 | **2.14** | **0.240** | NS | Loc; Refs |
| Among Refs | 3 | 50.82 | 16.94 | 15.98 | 0.001 | | |
| Sites (Loc(Bay)) | 10 | 20.49 | 2.05 | | | | Residuals |
| Sites (Farms) | 2 | 12.01 | 6.01 | 5.67 | 0.029 | SIG | Sites (Refs) |
| Sites (Refs) | 8 | 8.48 | 1.06 | 1.25 | 0.284 | | |
| Locations x Time | 36 | 172.03 | 4.78 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 51.46 | 5.72 | **1.28** | **0.292** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 120.57 | 4.47 | 5.26 | <0.000 | | |
| Sites(Loc) x Time | 90 | 88.17 | 0.98 | | | | Residuals |
| Sites (Farms) x Time | 18 | 27.04 | 1.50 | 1.77 | 0.047 | SIG | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 61.13 | 0.85 | 2.29 | <0.000 | | |
| Residuals | 300 | 111.32 | 0.37 | | | | |
| Total | 449 | 682.86 | | | | | |

**Table A3.12.** ANOVA results comparing the %N in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 0.1743 | 0.0194 | | | | Loc x Time |
| Locations | 4 | 0.0335 | 0.0084 | 93.06 | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.0284 | 0.0284 | **16.71** | **0.026** | SIG | Loc; Refs |
| Among Refs | 3 | 0.0051 | 0.0017 | 17.00 | 0.001 | | |
| Sites (Loc(Bay)) | 10 | 0.0009 | 0.0001 | 1.32 | 0.220 | | Residuals |
| Sites (Farms) | 2 | 0.0001 | 0.0001 | 0.50 | 0.624 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.0008 | 0.0001 | 0.64 | 0.739 | | |
| Locations x Time | 36 | 0.0541 | 0.0015 | 9.80 | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | **9** | 0.0066 | 0.0007 | **0.42** | **0.915** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 0.0475 | 0.0018 | 11.31 | <0.000 | | |
| Sites(Loc) x Time | 90 | 0.0138 | 0.0002 | 2.24 | <0.000 | | Residuals |
| Sites (Farms) x Time | 18 | 0.0026 | 0.0001 | 0.93 | 0.548 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 0.0112 | 0.0002 | 2.28 | <0.000 | | |
| Residuals | 300 | 0.0205 | 0.0001 | | | | |
| Total | 449 | | | | | | |

**Table A3.13.** ANOVA results comparing the %C in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with

three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 0.55 | 0.06 | | | | Loc x Time |
| Locations | 4 | 0.04 | 0.01 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.01 | 0.01 | **0.55** | **0.513** | NS | Loc; Refs |
| Among Refs | 3 | 0.04 | 0.01 | 1.94 | 0.202 | | |
| Sites (Loc(Bay)) | 10 | 0.05 | 0.01 | | | | Residuals |
| Sites (Farms) | 2 | 0.00 | 0.00 | 0.31 | 0.744 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.05 | 0.01 | 1.03 | 0.422 | | |
| Locations x Time | 36 | 0.62 | 0.02 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 0.12 | 0.01 | **0.71** | **0.693** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 0.50 | 0.02 | 3.10 | <0.000 | | |
| Sites(Loc) x Time | 90 | 0.45 | 0.01 | | | | Residuals |
| Sites (Farms) x Time | 18 | 0.02 | 0.00 | 0.19 | 1.000 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 0.43 | 0.01 | 3.96 | 1.000 | | |
| Residuals | 300 | 0.46 | 0.00 | | | | |
| Total | 449 | 2.18 | | | | | |

**Table A3.14.** ANOVA results comparing the P content in the soft sediments at Port George over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 1.37 | 0.17 | | | | Loc x Time |
| Locations | 4 | 0.98 | 0.25 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.65 | 0.65 | **5.81** | **0.095** | NS | Loc; Refs |
| Among Refs | 3 | 0.33 | 0.11 | 8.35 | 0.008 | | |
| Sites (Loc(Bay)) | 10 | 0.14 | 0.01 | | | | Residuals |
| Sites (Farms) | 2 | 0.03 | 0.02 | 1.18 | 0.356 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.11 | 0.01 | 1.27 | 0.273 | | |
| Locations x Time | 32 | 3.68 | 0.11 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.92 | 0.11 | **1.00** | **0.463** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 2.76 | 0.11 | 10.98 | 0.000 | | |
| Sites(Loc) x Time | 80 | 0.80 | 0.01 | | | | Residuals |
| Sites (Farms) x Time | 16 | 0.13 | 0.01 | 0.77 | 0.708 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.67 | 0.01 | 1.30 | 0.082 | | |
| Residuals | 270 | 2.18 | 0.01 | | | | |
| Total | 404 | 9.15 | 0.02 | | | | |

**Table A3.15.** ANOVA results comparing the %Carbonates in the soft sediments at Port George over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 268.68 | 29.853 | | | | Loc x Time |
| Locations | 4 | 67.22 | 16.806 | 4.55 | 0.024 | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 38.80 | 38.802 | **4.10** | **0.136** | NS | Loc; Refs |
| Among Refs | 3 | 28.43 | 9.474 | 2.24 | 0.161 | | |
| Sites (Loc(Bay)) | 10 | 36.90 | 3.690 | 2.23 | 0.016 | | Residuals |
| Sites (Farms) | 2 | 3.09 | 1.549 | 0.37 | 0.704 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 33.81 | 4.226 | 0.65 | 0.731 | | |
| Locations x Time | 36 | 384.56 | 10.682 | 1.68 | 0.025 | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 108.01 | 12.001 | **1.17** | **0.351** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 276.55 | 10.243 | 1.58 | 0.064 | | |
| Sites(Loc) x Time | 90 | 571.34 | 6.348 | | | | Residuals |
| Sites (Farms) x Time | 18 | 104.82 | 5.823 | 0.90 | 0.582 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 466.53 | 6.480 | 3.91 | <0.000 | | |
| Residuals | 300 | 496.95 | 1.656 | | | | |
| Total | 449 | 1825.67 | | | | | |

**Table A3.16.** ANOVA results comparing the Redox potential of the soft sediments at Port George over eight different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 7 | 224115 | 32016 | | | | Loc x Time |
| Locations | 4 | 72104 | 18026 | 13.59 | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 31622 | 31622 | **2.34** | **0.223** | NS | Loc; Refs |
| Among Refs | 3 | 40482 | 13494 | 8.25 | 0.008 | | |
| Sites (Loc(Bay)) | 10 | 13265 | 1326 | 1.00 | 0.443 | | Residuals |
| Sites (Farms) | 2 | 335 | 168 | 0.10 | 0.903 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 12930 | 1616 | 0.62 | 0.759 | | |
| Locations x Time | 28 | 136743 | 4884 | 2.05 | 0.008 | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 7 | 9108 | 1301 | **0.21** | **0.978** | NS | Loc;Refs x Ti |
| Among Refs x Time | 21 | 127635 | 6078 | 2.32 | 0.006 | | |
| Sites(Loc) x Time | 70 | 166760 | 2382 | 1.80 | 0.001 | | Residuals |
| Sites (Farms) x Time | 14 | 20205 | 1443 | 0.55 | 0.890 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 56 | 146555 | 2617 | 1.98 | <0.000 | | |
| Residuals | 240 | 317913 | 1325 | | | | |
| Total | 359 | 930901 | 2593 | | | | |

## VANSITTART BAY ANOVAS

**Table A3.17.** ANOVA results comparing the number of benthic macrofauna taxa in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 844.76 | 93.86 | | | | Loc x Time |
| Locations | 4 | 779.52 | 194.88 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 172.36 | 172.36 | **0.85** | **0.424** | NS | Loc; Refs |
| Among Refs | 3 | 607.16 | 202.39 | 4.60 | 0.038 | | |
| Sites (Loc(Bay)) | 10 | 388.22 | 38.82 | | | | Residuals |
| Sites (Farms) | 2 | 36.02 | 18.01 | 0.41 | 0.677 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 352.20 | 44.03 | 3.07 | 0.005 | | |
| Locations x Time | 36 | 528.25 | 14.67 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 119.06 | 13.23 | **1.02** | **0.431** | NS | Ti x Site (Loc) |
| Among Refs x Time | 27 | 409.20 | 15.16 | 1.06 | 0.413 | | |
| Sites(Loc) x Time | 90 | 1168.22 | 12.98 | | | | Residuals |
| Sites (Farms) x Time | 18 | 134.87 | 7.49 | 0.52 | 0.939 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 1033.36 | 14.35 | 2.38 | <0.000 | | |
| Residuals | 300 | 1812.00 | 6.04 | | | | |
| Total | 449 | 5520.98 | | | | | |

**Table A3.18.** ANOVA results comparing the number of benthic macrofauna individuals in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 69.32 | 7.70 | | | | Loc x Time |
| Locations | 4 | 72.28 | 18.07 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 29.10 | 29.10 | **2.02** | **0.250** | NS | Loc; Refs |
| Among Refs | 3 | 43.18 | 14.39 | 3.66 | 0.063 | | |
| Sites (Loc(Bay)) | 10 | 34.04 | 3.40 | | | | Residuals |
| Sites (Farms) | 2 | 2.59 | 1.29 | 0.33 | 0.729 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 31.46 | 3.93 | 5.92 | <0.000 | | |
| Locations x Time | 36 | 55.98 | 1.55 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 10.94 | 1.22 | **0.73** | **0.679** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 45.04 | 1.67 | 2.51 | 0.001 | | |
| Sites(Loc) x Time | 90 | 56.48 | 0.63 | | | | Residuals |
| Sites (Farms) x Time | 18 | 8.70 | 0.48 | 0.73 | 0.772 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 47.79 | 0.66 | 2.12 | <0.000 | | |
| Residuals | 300 | 94.07 | 0.31 | | | | |
| Total | 449 | 382.17 | | | | | |

**Table A3.19.** ANOVA results comparing the %TOM in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 223.85 | 24.87 | | | | Loc x Time |
| Locations | 4 | 372.28 | 93.07 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 61.14 | 61.14 | **0.59** | **0.499** | NS | Loc; Refs |
| Among Refs | 3 | 311.14 | 103.71 | 12.18 | 0.002 | | |
| Sites (Loc(Bay)) | 10 | 86.85 | 8.68 | | | | Residuals |
| Sites (Farms) | 2 | 18.73 | 9.37 | 1.10 | 0.378 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 68.12 | 8.51 | 5.75 | <0.000 | | |
| Locations x Time | 36 | 210.61 | 5.85 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 43.73 | 4.86 | **0.79** | **0.631** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 166.88 | 6.18 | 4.17 | <0.000 | | |
| Sites(Loc) x Time | 90 | 140.40 | 1.56 | | | | Residuals |
| Sites (Farms) x Time | 18 | 33.80 | 1.88 | 1.27 | 0.235 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 106.60 | 1.48 | 2.69 | <0.000 | | |
| Residuals | 300 | 165.14 | 0.55 | | | | |
| Total | 449 | 1199.13 | | | | | |

**Table A3.20.** ANOVA results comparing the %N in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 0.303 | 0.034 | | | | Loc x Time |
| Locations | 4 | 0.050 | 0.012 | 6.53 | 0.008 | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.000 | 0.000 | **0.02** | **0.901** | NS | Loc; Refs |
| Among Refs | 3 | 0.050 | 0.017 | 6.96 | 0.013 | | |
| Sites (Loc(Bay)) | 10 | 0.019 | 0.002 | 16.71 | 0.000 | | Residuals |
| Sites (Farms) | 2 | 0.000 | 0.000 | 0.02 | 0.979 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.019 | 0.002 | 8.26 | <0.000 | | |
| Locations x Time | 36 | 0.027 | 0.001 | 2.67 | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 0.013 | 0.001 | **2.55** | **0.029** | Sig | Loc;Refs x Ti |
| Among Refs x Time | 27 | 0.015 | 0.001 | 1.91 | 0.016 | | |
| Sites(Loc) x Time | 90 | 0.026 | 0.000 | 2.50 | | | Residuals |
| Sites (Farms) x Time | 18 | 0.005 | 0.000 | 0.97 | 0.507 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 0.021 | 0.000 | 2.51 | <0.000 | | |
| Residuals | 300 | 0.034 | 0.000 | | | | |
| Total | 449 | 0.460 | | | | | |

**Table A3.21.** ANOVA results comparing the %C in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with

three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 2.570 | 0.286 | | | | Loc x Time |
| Locations | 4 | 1.677 | 0.419 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 1.206 | 1.206 | **7.67** | **0.070** | NS | Loc; Refs |
| Among Refs | 3 | 0.472 | 0.157 | 1.78 | 0.229 | | |
| Sites (Loc(Bay)) | 10 | 0.710 | 0.071 | | | | Residuals |
| Sites (Farms) | 2 | 0.002 | 0.001 | 0.01 | 0.989 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.708 | 0.088 | 12.96 | <0.000 | | |
| Locations x Time | 36 | 2.178 | 0.061 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 0.583 | 0.065 | **1.10** | **0.398** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 1.595 | 0.059 | 8.60 | <0.000 | | |
| Sites(Loc) x Time | 90 | 0.511 | 0.006 | | | | Residuals |
| Sites (Farms) x Time | 18 | 0.020 | 0.001 | 0.16 | 1.000 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 0.492 | 0.007 | 6.17 | <0.000 | | |
| Residuals | 300 | 0.332 | 0.001 | | | | |
| Total | 449 | 7.978 | | | | | |

**Table A3.22.** ANOVA results comparing the P content in the soft sediments at Vansittart Bay over nine different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 8 | 2.18 | 0.27 | | | | Loc x Time |
| Locations | 4 | 0.30 | 0.08 | | | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 0.00 | 0.00 | **0.01** | **0.911** | NS | Loc; Refs |
| Among Refs | 3 | 0.30 | 0.10 | 3.57 | 0.067 | | |
| Sites (Loc(Bay)) | 10 | 0.28 | 0.03 | | | | Residuals |
| Sites (Farms) | 2 | 0.06 | 0.03 | 1.02 | 0.405 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.23 | 0.03 | 2.13 | 0.046 | | |
| Locations x Time | 32 | 1.87 | 0.06 | | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 8 | 0.79 | 0.10 | **2.17** | **0.068** | NS | Loc;Refs x Ti |
| Among Refs x Time | 24 | 1.09 | 0.05 | 3.40 | <0.000 | | |
| Sites(Loc) x Time | 80 | 0.91 | 0.01 | | | | Residuals |
| Sites (Farms) x Time | 16 | 0.06 | 0.00 | 0.27 | 0.997 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 64 | 0.85 | 0.01 | 2.05 | <0.000 | | |
| Residuals | 270 | 1.75 | 0.01 | | | | |
| Total | 404 | 7.30 | 0.02 | | | | |

**Table A3.23.** ANOVA results comparing the %Carbonates in the soft sediments at Vansittart Bay over ten different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 9 | 13.96 | 1.55 | | | | Loc x Time |
| Locations | 4 | 11.95 | 2.99 | 7.98 | 0.004 | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 2.54 | 2.54 | **0.81** | **0.435** | NS | Loc; Refs |
| Among Refs | 3 | 9.41 | 3.14 | 6.98 | 0.013 | | |
| Sites (Loc(Bay)) | 10 | 3.74 | 0.37 | 15.82 | <0.000 | | Residuals |
| Sites (Farms) | 2 | 0.15 | 0.07 | 0.16 | 0.852 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 3.60 | 0.45 | 8.20 | 0.000 | | |
| Locations x Time | 36 | 7.91 | 0.22 | 4.46 | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 9 | 0.81 | 0.09 | **0.34** | **0.953** | NS | Loc;Refs x Ti |
| Among Refs x Time | 27 | 7.10 | 0.26 | 4.80 | <0.000 | | |
| Sites(Loc) x Time | 90 | 4.43 | 0.05 | 2.08 | | | Residuals |
| Sites (Farms) x Time | 18 | 0.48 | 0.03 | 0.49 | 0.956 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 72 | 3.95 | 0.05 | 2.32 | <0.000 | | |
| Residuals | 300 | 7.10 | 0.02 | | | | |
| Total | 449 | 49.09 | | | | | |

**Table A3.24.** ANOVA results comparing the Redox potential of the soft sediments at Vansittart Bay over eight different sampling times. The farm was compared to four reference locations and with three sites nested in each of these locations. NS = No significant difference. Homogeneity of variances was not achieved after transforming the data.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested over |
|---|---|---|---|---|---|---|---|
| Time | 7 | 6.808 | 0.973 | | | | Loc x Time |
| Locations | 4 | 5.680 | 1.420 | 16.629 | 0.000 | | Sites (Loc) |
| **Farms vs. Refs** | 1 | 2.274 | 2.274 | **2.002** | **0.252** | NS | Loc; Refs |
| Among Refs | 3 | 3.407 | 1.136 | 10.80 | 0.003 | | |
| Sites (Loc(Bay)) | 10 | 0.854 | 0.085 | 5.703 | <0.000 | | Residuals |
| Sites (Farms) | 2 | 0.013 | 0.007 | 0.062 | 0.940 | NS | Sites (Refs) |
| Sites (Refs) | 8 | 0.841 | 0.105 | 3.89 | 0.001 | | |
| Locations x Time | 28 | 3.350 | 0.120 | 4.976 | | | Sites(Loc)x Ti |
| **Farm vs. Refs x Time** | 7 | 0.502 | 0.072 | **0.528** | **0.803** | NS | Loc;Refs x Ti |
| Among Refs x Time | 21 | 2.849 | 0.136 | 5.02 | <0.000 | | |
| Sites(Loc) x Time | 70 | 1.683 | 0.024 | 1.606 | 0.005 | | Residuals |
| Sites (Farms) x Time | 14 | 0.170 | 0.012 | 0.449 | 0.950 | NS | Sites(Refs) x Ti |
| Sites (Refs) x Time | 56 | 1.513 | 0.027 | 1.805 | 0.001 | | |
| Residuals | 240 | 3.594 | 0.015 | | | | |
| Total | 359 | 21.970 | 0.061 | | | | |

## APPENDIX FOUR; ANOVAS FOR THE BACI STUDY

**Table A4.1.** ANOVA results comparing the number of macrofauna taxa (per $10m^2$) in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS

= No significant difference. In bold are the values that compare farms with reference locations.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 330.70 | 165.35 | | | | |
| Time (Period) | 3 | 607.17 | 202.39 | | | | |
| Location | 3 | 59.04 | 19.68 | | | | |
| Farms vs. Refs | (1) | 54.54 | 54.54 | 1.72 | 0.202 | NS | Ti(Per) x Site(loc) |
| Among Refs | (2) | 4.49 | 2.25 | 0.24 | 0.801 | | Per x Among Refs |
| Sites (Locations) | 8 | 99.33 | 12.42 | 0.62 | 0.755 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 22.37 | 11.19 | 0.72 | 0.542 | NS | Per x Sites(farms) |
| Sites (Refs) | (6) | 76.96 | 12.83 | 0.87 | 0.533 | | Per x Sites (Refs) |
| Period X Location | 6 | 226.63 | 37.77 | | | | |
| **Period X Farms vs. Refs** | (2) | 18.38 | 9.19 | **0.18** | **0.844** | **NS** | Per x Among Refs |
| Period X Among Refs | (4) | 208.25 | 52.06 | 6.20 | 0.006 | | Per x Sites (Refs) |
| Period X Sites (Loc) | 16 | 163.33 | 10.21 | 0.51 | 0.918 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 62.52 | 15.63 | 1.86 | 0.182 | NS | Per x Sites(Refs) |
| Period X Sites (Refs) | (12) | 100.81 | 8.40 | 6.20 | 0.006 | | Res |
| Time(Period) X Locations | 9 | 242.83 | 26.98 | 1.34 | 0.268 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs. Refs** | (3) | 77.20 | 25.73 | **0.93** | **0.481** | **NS** | Ti(Per) x Among Ref |
| Time(Per) X Among Refs | (6) | 165.63 | 27.60 | 1.88 | 0.140 | | Ti(Per) x Site(Refs) |
| Time (Per) X Sites (Loc) | 24 | 482.67 | 20.11 | 1.85 | 0.014 | | Res |
| Time(Per) X Sites (Farms) | (6) | 218.44 | 36.41 | 2.48 | 0.063 | NS | Ti(Per) x Site(Refs) |
| Time(Per) X Sites (Refs) | (18) | 264.22 | 14.68 | 1.35 | 0.166 | | Res |
| Residuals | 144 | 1564.67 | 10.87 | | | | |
| Total | 215 | 3776.37 | | | | | |

**Table A4.2.** ANOVA results comparing the number of macrofauna individuals (per $10m^2$) in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS = No significant difference. The data was transformed ln(x+1) to achieve homogeneity of variances.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 6.77 | 3.39 | | | | |
| Time (Period) | 3 | 17.28 | 5.76 | | | | |
| Location | 3 | 2.02 | 0.67 | | | | |
| Farms vs. Refs | (1) | 1.19 | 1.19 | 2.89 | 0.231 | NS | Loc (Among Refs) |
| Among Refs | (2) | 0.82 | 0.41 | 2.37 | 0.174 | | Per x Among Refs |
| Sites (Locations) | 8 | 1.28 | 0.16 | 0.31 | 0.956 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 0.24 | 0.12 | 0.33 | 0.735 | NS | Per x Sites(farms) |
| Sites (Refs) | (6) | 1.04 | 0.17 | 0.45 | 0.837 | | Ti(Per)x Sites (Refs) |
| Period X Location | 6 | 6.47 | 1.08 | | | | |
| **Period X Farms vs. Refs** | (2) | 3.17 | 1.58 | **1.92** | **0.260** | **NS** | Per x Among Refs |
| Period X Among Refs | (4) | 3.30 | 0.82 | 2.78 | 0.076 | | Ti(Per)x Among Refs |
| Period X Sites (Loc) | 16 | 4.98 | 0.31 | 0.60 | 0.854 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 1.43 | 0.36 | 1.20 | 0.359 | NS | Per x Sites(Refs) |
| Period X Sites (Refs) | (12) | 3.56 | 0.30 | 0.77 | 0.677 | | |
| Time(Period) X Locations | 9 | 5.83 | 0.65 | 1.25 | 0.315 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs. Refs** | (3) | 1.33 | 0.44 | **0.59** | **0.644** | **NS** | Ti(Per)x Among Refs |
| Time(Per) X Among Refs | (6) | 4.50 | 0.75 | 1.94 | 0.129 | | Ti(Per)x Site (Refs) |
| Time (Per) X Sites (Loc) | 24 | 12.48 | 0.52 | 1.79 | 0.020 | | Res |
| Time(Per) X Sites (Farms) | (6) | 5.51 | 0.92 | 2.37 | 0.073 | NS | Ti(Per)x Site (Refs) |
| Time(Per) X Sites (Refs) | (18) | 6.97 | 0.39 | 1.33 | 0.177 | | |
| Residuals | 144 | 41.91 | 0.29 | | | | |
| Total | 215 | 99.01 | | | | | |

**Table A4.3.** ANOVA results comparing the percentage of Total Organic Matter (TOM) in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 85.39 | 42.70 | | | | |
| Time (Period) | 3 | 447.65 | 149.22 | | | | |
| Location | 3 | 13.60 | 4.53 | | | | |
| Farms vs. Refs | (1) | 0.44 | 0.44 | 0.09 | 0.771 | NS | Per x Locs |
| Among Refs | (2) | 13.16 | 6.58 | 0.95 | 0.459 | | Per x Among Refs |
| Sites (Locations) | 8 | 8.65 | 1.08 | 1.63 | 0.170 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 6.96 | 3.48 | 3.44 | 0.135 | NS | Per x Sites(farms) |
| Sites (Refs) | (6) | 1.69 | 0.28 | 0.36 | 0.893 | | Per x Sites (Refs) |
| Period X Location | 6 | 28.59 | 4.76 | | | | |
| **Period X Farms vs. Refs** | (2) | 0.96 | 0.48 | **0.07** | **0.934** | **NS** | Per x Among Refs |
| Period X Among Refs | (4) | 27.63 | 6.91 | 8.74 | 0.002 | | Ti(Per)x Site(Refs) |
| Period X Sites (Loc) | 16 | 13.53 | 0.85 | 1.27 | 0.290 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 4.04 | 1.01 | 1.28 | 0.232 | NS | Per x Sites (Refs) |
| Period X Sites (Refs) | (12) | 9.49 | 0.79 | 2.41 | 0.007 | | Res |
| Time(Period) X Locations | 9 | 39.88 | 4.43 | 6.66 | <0.000 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs.Refs** | (3) | 20.53 | 6.84 | **2.12** | **0.199** | **NS** | Ti(Per)x Among Refs |
| Time(Per) X Among Refs | (6) | 19.35 | 3.22 | 9.83 | <0.000 | | Res |
| Time (Per) X Sites (Loc) | 24 | 15.96 | 0.66 | 2.03 | 0.006 | | Res |
| Time(Per) X Sites (Farms) | (6) | 8.98 | 1.50 | 4.56 | <0.000 | SIG | Res |
| Time(Per) X Sites (Refs) | (18) | 6.98 | 0.39 | 1.18 | 0.284 | | |
| Residuals | 144 | 47.24 | 0.33 | | | | |
| Total | 215 | 700.48 | | | | | |

**Table A4.4.** ANOVA results comparing the percentage of nitrogen in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 0.0551 | 0.0276 | | | | |
| Time (Period) | 3 | 0.0336 | 0.0112 | | | | |
| Location | 3 | 0.0084 | 0.0028 | | | | |
| Farms vs. Refs | (1) | 0.0044 | 0.0044 | 2.20 | 0.276 | NS | Locs Among Refs |
| Among Refs | (2) | 0.0040 | 0.0020 | 2.96 | 0.162 | | Per x Among Refs |
| Sites (Locations) | 8 | 0.0020 | 0.0003 | 1.71 | 0.146 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 0.0008 | 0.0004 | 2.00 | 0.216 | NS | Sites(Refs) |
| Sites (Refs) | (6) | 0.0012 | 0.0002 | 1.33 | 0.243 | | Per x Sites(Refs) |
| Period X Location | 6 | 0.0064 | 0.0011 | | | | |
| **Period X Farms vs. Refs** | (2) | 0.0037 | 0.0019 | **2.74** | **0.178** | **NS** | Per x Among Refs |
| Period X Among Refs | (4) | 0.0027 | 0.0007 | 4.50 | 0.011 | | Ti(Per)x Site(Refs) |
| Period X Sites (Loc) | 16 | 0.0017 | 0.0001 | 0.73 | 0.741 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 0.0002 | 0.0001 | 0.34 | 0.846 | NS | Per x Sites (Refs) |
| Period X Sites (Refs) | (12) | 0.0015 | 0.0001 | 0.83 | 0.619 | | Ti(Per) x Site(loc) |
| Time(Period) X Locations | 9 | 0.0126 | 0.0014 | 9.60 | <0.000 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs.Refs** | (3) | 0.0090 | 0.0030 | **5.00** | **0.045** | **SIG** | Ti(Per)x Among Refs |
| Time(Per) X Among Refs | (6) | 0.0036 | 0.0006 | 4.00 | 0.010 | | Ti(Per) x Sites(Refs) |
| Time (Per) X Sites (Loc) | 24 | 0.0035 | 0.0001 | 2.66 | <0.000 | | Res |
| Time(Per) X Sites (Farms) | (6) | 0.0008 | 0.0001 | 0.89 | 0.523 | NS | Ti(Per) x Sites(Refs) |
| Time(Per) X Sites (Refs) | (18) | 0.0027 | 0.0002 | 2.73 | <0.000 | | |
| Residuals | 144 | 0.0079 | 0.0001 | | | | |
| Total | 215 | 0.1312 | | | | | |

**Table A4.5.** ANOVA results comparing the percentage of carbon in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 5.48 | 2.74 | | | | |
| Time (Period) | 3 | 52.33 | 17.44 | 44.69 | 0.000 | | |
| Location | 3 | 7.01 | 2.34 | | | | |
| Farms vs. Refs | (1) | 1.43 | 1.43 | 0.51 | 0.548 | NS | Locs Among Refs |
| Among Refs | (2) | 5.57 | 2.79 | 8.84 | 0.002 | | Ti(Per) x Sites(Refs) |
| Sites (Locations) | 8 | 3.45 | 0.43 | 1.10 | 0.395 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 1.28 | 0.64 | 3.75 | 0.121 | NS | Per x Sites(Farms) |
| Sites (Refs) | (6) | 2.16 | 0.36 | 1.14 | 0.378 | | Ti(Per) x Sites(Refs) |
| Period X Location | 6 | 1.34 | 0.22 | | | | |
| **Period X Farms vs. Refs** | (2) | 0.88 | 0.44 | **2.16** | **0.147** | **NS** | Per x Site(loc) |
| Period X Among Refs | (4) | 0.46 | 0.11 | 0.53 | 0.714 | | Ti(Per)x Site(Refs) |
| Period X Sites (Loc) | 16 | 3.26 | 0.20 | 0.52 | 0.909 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 0.69 | 0.17 | 0.44 | 0.779 | NS | Ti(Per) x Sites(loc) |
| Period X Sites (Refs) | (12) | 2.57 | 0.21 | 0.68 | 0.750 | | Ti(Per) x Site(Refs) |
| Time(Period) X Locations | 9 | 2.80 | 0.31 | 0.80 | 0.622 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs. Refs** | (3) | 1.92 | 0.64 | **4.35** | **0.060** | **NS** | Ti(Per) x Sites(loc) |
| Time(Per) X Among Refs | (6) | 0.88 | 0.15 | 0.47 | 0.824 | | Ti(Per) x Sites(Refs) |
| Time (Per) X Sites (Loc) | 24 | 9.37 | 0.39 | 8.09 | <0.000 | | Res |
| Time(Per) X Sites (Farms) | (6) | 3.69 | 0.62 | 1.95 | 0.127 | NS | Ti(Per) x Sites(Refs) |
| Time(Per) X Sites (Refs) | (18) | 5.67 | 0.32 | 6.54 | <0.000 | | Res |
| Residuals | 144 | 6.94 | 0.05 | | | | |
| Total | 215 | 91.96 | | | | | |

**Table A4.6.** ANOVA results comparing the percentage of carbonates in soft sediments; before, one year after and two years after the closure of a pearl farm (i.e. three periods). There were two sampling times in each period, during which, one farm location was compared to three reference locations. Three sites were nested in each of these locations. NS = No significant difference.

| Source of Variation | DF | SS | MS | F | *P* | Sig | Tested Over |
|---|---|---|---|---|---|---|---|
| Period | 2 | 14.92 | 7.46 | | | | |
| Time (Period) | 3 | 88.71 | 29.57 | 6.22 | 0.003 | | |
| Location | 3 | 98.54 | 32.85 | | | | |
| Farms vs. Refs | (1) | 31.87 | 31.87 | 0.96 | 0.431 | NS | Locs Among Refs |
| Among Refs | (2) | 66.67 | 33.34 | 9.89 | 0.028 | | Per x Among Refs |
| Sites (Locations) | 8 | 130.97 | 16.37 | 3.44 | 0.009 | | Ti(Per) x Site(loc) |
| Sites (Farms) | (2) | 109.44 | 54.72 | 40.83 | 0.002 | SIG | Per x Sites(Farms) |
| Sites (Refs) | (6) | 21.53 | 3.59 | 1.67 | 0.322 | | Ti(Per) x Sites(Refs) |
| Period X Location | 6 | 51.28 | 8.55 | | | | |
| **Period X Farms vs. Refs** | (2) | 37.80 | 18.90 | **5.61** | **0.069** | **NS** | Per x Among Refs |
| Period X Among Refs | (4) | 13.48 | 3.37 | 1.57 | 0.245 | | Ti(Per)x Site(Refs) |
| Period X Sites (Loc) | 16 | 31.09 | 1.94 | 0.41 | 0.966 | | Ti(Per) x Site(loc) |
| Period X Sites (Farms) | (4) | 5.36 | 1.34 | 0.28 | 0.887 | NS | Ti(Per) x Sites(loc) |
| Period X Sites (Refs) | (12) | 25.73 | 2.14 | 0.70 | 0.729 | | Ti(Per) x Site(Refs) |
| Time(Period) X Locations | 9 | 125.19 | 13.91 | 2.92 | 0.017 | | Ti(Per) x Site(loc) |
| **Time(Per) X Farm vs. Refs** | (3) | 81.71 | 27.24 | **3.76** | **0.079** | **NS** | Ti(Per) x Among Refs |
| Time(Per) X Among Refs | (6) | 43.47 | 7.25 | 2.38 | 0.072 | | Ti(Per) x Sites(Refs) |
| Time (Per) X Sites (Loc) | 24 | 114.13 | 4.76 | 2.24 | 0.002 | | Res |
| Time(Per) X Sites (Farms) | (6) | 59.29 | 9.88 | 3.24 | 0.024 | SIG | Ti(Per) x Sites(Refs) |
| Time(Per) X Sites (Refs) | (18) | 54.84 | 3.05 | 1.43 | 0.124 | | |
| Residuals | 144 | 306.05 | 2.13 | | | | |
| Total | 215 | 960.87 | | | | | |


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